

PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

| | | | |
|--|--|--|---|
| <p>(51) International Patent Classification 6 : G01N 33/48, C07H 21/04</p> | | <p>A1</p> | <p>(11) International Publication Number: WO 98/27425</p> <p>(43) International Publication Date: 25 June 1998 (25.06.98)</p> |
| <p>(21) International Application Number: PCT/US97/23284</p> <p>(22) International Filing Date: 18 December 1997 (18.12.97)</p> <p>(30) Priority Data: 08/769,951 19 December 1996 (19.12.96) US</p> <p>(71) Applicant (for all designated States except US): ISIS PHARMACEUTICALS, INC. [US/US]; 2292 Faraday Avenue, Carlsbad, CA 92008 (US).</p> <p>(72) Inventors; and (75) Inventors/Applicants (for US only): CHEN, Danhua [CN/US]; 1442 Via Terrassa, Encinitas, CA 92024 (US). SRIVATSA, Githa, Susan [US/US]; 1642 Orchard Wood Road, Encinitas, CA 92024 (US). COLE, Douglas, L. [US/US]; 4992 Smith Canyon Court, San Diego, CA 92130 (US).</p> <p>(74) Agents: CALDWELL, John, W. et al.; Woodcock Washburn Kurtz Mackiewicz & Norris LLP, 46th floor, One Liberty Place, Philadelphia, PA 19103 (US).</p> | | <p>(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KO, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KR, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).</p> <p>Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p> | |
| <p>(54) Title: LARGE-SCALE PURIFICATION OF FULL LENGTH OLIGONUCLEOTIDES BY SOLID-LIQUID AFFINITY EXTRACTION</p> <p>(57) Abstract</p> <p>Methods and matrices are provided for purifying a desired target oligonucleotide using an immobilized affinity unit that selectively binds the target oligonucleotide. The immobilized affinity unit preferably comprises a nucleobase sequence which comprises the reverse complement of the target oligonucleotide. Preferred methods of the invention result in the rapid, cost-effective and efficacious separation of most undesired contaminants, particularly undesired deletion [e.g., (n-1), (n-2), etc.] derivatives of the desired full length (n) oligonucleotide that result from incomplete oligomerization or through degradative processes, including those undesired derivatives having internal or 3' deletions of one or more nucleotides.</p> | | | |

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

| | | | | | | | |
|----|--------------------------|----|---------------------|----|-----------------------|----|--------------------------|
| AL | Albania | ES | Spain | LS | Lesotho | SI | Slovenia |
| AM | Armenia | FI | Finland | LT | Lithuania | SK | Slovakia |
| AT | Austria | FR | France | LU | Luxembourg | SN | Senegal |
| AU | Australia | GA | Gabon | LV | Latvia | SZ | Swaziland |
| AZ | Azerbaijan | GB | United Kingdom | MC | Monaco | TD | Chad |
| BA | Bosnia and Herzegovina | GE | Georgia | MD | Republic of Moldova | TG | Togo |
| BB | Barbados | GH | Ghana | MG | Madagascar | TJ | Tajikistan |
| BE | Belgium | GN | Guinea | MK | The former Yugoslav | TM | Turkmenistan |
| BF | Burkina Faso | GR | Greece | MR | Republic of Macedonia | TR | Turkey |
| BG | Bulgaria | HU | Hungary | ML | Mali | TT | Trinidad and Tobago |
| BJ | Benin | IE | Ireland | MN | Mongolia | UA | Ukraine |
| BR | Brazil | IL | Israel | MR | Mariniania | UG | Uganda |
| BY | Belarus | IS | Iceland | MW | Maliawi | US | United States of America |
| CA | Canada | IT | Italy | MX | Mexico | UZ | Uzbekistan |
| CF | Central African Republic | JP | Japan | NE | Niger | VN | Viet Nam |
| CG | Coego | KE | Kenya | NL | Netherlands | YU | Yugoslavia |
| CH | Switzerland | KG | Kyrgyzstan | NO | Norway | ZW | Zimbabwe |
| CI | Côte d'Ivoire | KP | Democratic People's | NZ | New Zealand | | |
| CM | Cameroon | | Republic of Korea | PL | Poland | | |
| CN | China | KR | Republic of Korea | PT | Portugal | | |
| CU | Cuba | KZ | Kazakhstan | RO | Romania | | |
| CZ | Czech Republic | LC | Saint Lucia | RU | Russian Federation | | |
| DE | Germany | LI | Liechtenstein | SD | Sudan | | |
| DK | Denmark | LK | Sri Lanka | SE | Sweden | | |
| EE | Estonia | LR | Liberia | SG | Singapore | | |

LARGE-SCALE PURIFICATION OF FULL LENGTH
OLIGONUCLEOTIDES BY SOLID-LIQUID
AFFINITY EXTRACTION

CROSS REFERENCE TO RELATED APPLICATIONS

5 This application is a continuation in part of
Serial No. 08/769,951 filed December 19, 1996.

FIELD OF THE INVENTION

This invention relates to methods for purifying a desired full length synthetic oligonucleotide (the target oligonucleotide) from a mixture containing undesired contaminants (e.g., deletion derivatives of the target oligonucleotide) using an immobilized affinity unit that selectively and reversibly binds the target oligonucleotide. Under appropriately stringent conditions, hybridization 10 between the target oligonucleotide and the affinity unit, which comprises a nucleobase sequence having reverse complementary to the nucleobase sequence of at least a central portion of the target oligonucleotide, results in the selective retention of the desired full length (n) 15 target oligonucleotide. Undesired derivatives (e.g., all forms of (n-1), (n-2), etc.) of the target oligonucleotide that result from, e.g., incomplete oligomerization during synthesis or degradative processes either fail to hybridize, 20 and are not so retained, or hybridize with a lesser affinity

- 2 -

than the target oligonucleotide and may thus be removed by treatments (e.g., changes in pH, ionic strength, temperature, and the like) which do not significantly effect the hybridization between the affinity unit and the target 5 oligonucleotide. The desired full length (n) target oligonucleotide is thus rapidly, cost-effectively and efficaciously separated from undesired contaminants including the undesired derivatives (e.g., all forms of (n-1), (n-2), etc.) of the target oligonucleotide that result 10 from, e.g., incomplete oligomerization during synthesis or degradative processes. Preferred matrices for practicing the methods of the invention are also herein provided.

BACKGROUND OF THE INVENTION

During chemical oligonucleotide synthesis, a 15 series of nucleoside monomers are sequentially attached to each other in a predetermined order so that an oligonucleotide having a desired sequence is obtained. Each nucleoside monomer is attached to the growing chain sequentially through a series of chemical reactions or 20 "steps." For example, a process of oligonucleotide synthesis generally comprises the steps of (1) blocking chemically reactive sites on the base portion of a first and second selected nucleoside with unreactive "blocking groups," (2) coupling the first selected base-blocked 25 nucleoside monomer to an inorganic support via a 3' hydroxyl linkage from the pentose portion of the first nucleoside monomer, (3) "protecting" the 5' hydroxyl position of the pentose portion of the second selected base-blocked nucleoside monomer, for example, by chemically attaching a 30 dimethoxytrityl (DMTr) group thereto, (4) attaching the second selected base- and 5'-blocked nucleoside monomer via a linkage spanning the 5' carbon of the pentose portion of the first selected base-blocked nucleoside monomer and the 3' carbon of the pentose portion of the second selected 35 base- and 5'-blocked nucleoside monomer, (5) acylating or otherwise "capping" unreacted nucleoside monomers and (6)

- 3 -

"deprotecting" (e.g., detrityling) the linked nucleosides, i.e., removing the DMTr or other protecting group attached to the 5' hydroxyl position of the pentose portion of the second selected base-blocked nucleoside monomer in order to 5 regenerate a reactive site for a second cycle of such steps. Upon completion of a desired number of such cycles, the oligonucleotide is "deblocked" (i.e., the blocking groups attached to the bases of the oligonucleotide are removed) and a desired biological activity is then realized.

10 Such methods of oligonucleotide synthesis include, for example, those commonly known as the "phosphite triester method," the "phosphotriester method" and the "H-phosphonate method." Methods for the solution phase synthesis of oligonucleotides have also been described (see U.S. Patent 15 No. 5,210,264 to Yau, assigned to the present applicants; Reese et al., *J. Chem. Soc. Perkin Trans.*, 1993, 1, 2291; and Wada et al., *Tetrahedron*, 1993, 49, 2043). Regardless of which method is used, however, the stepwise yield for each nucleoside addition is typically about 99%. Thus, 20 approximately 1% of the oligomers fail nucleoside monomer addition in each step. Such failures of addition result from incomplete coupling, incomplete capping, incomplete detritylation, undesired retritylation through oxidation or by other mechanisms. For a desired synthetic 25 oligonucleotide product of length n, the resulting oligonucleotides are, e.g., one oligonucleotide (n-1), two oligonucleotides (n-2), etc. shorter in length than the desired oligonucleotide and are present as undesired impurities. Such shorter, undesired oligonucleotides are 30 commonly and collectively referred to as "deletion sequences" or "failure" or "failed" sequences.

At one time, it was believed in the art that the majority of the failed sequences resulted from the "deletion" (or lack of incorporation) of nucleotides present 35 at the 5' end of the desired oligonucleotide. See, for example, U.S. Patent No. 5,352,578 to Agrawal et al., which issued October 4, 1994 (column 1, lines 56-59). However,

- 4 -

Temsamani et al. (*Nucl. Acids Res.*, 1995, 23, 1841) describe experiments in which the sequence identities of (n-1) [and some (n-2) and (n-3)] derivatives of a target full length (n=25 nucleotides) oligonucleotide were determined. Two 5 significant results emerged from these experiments. First, the (n-1) population of sequences was heterogeneous. The second finding, described as a "surprising result," was that there was a high frequency of sequences truncated at the 3' terminus and no deletions within the four most 5' terminal 10 nucleotides. Thus, a need exists for a method of purifying a desired full length synthetic oligonucleotide from a heterogeneous mixture containing "failed" sequence oligonucleotides, wherein deletions (failures of incorporation) are randomly distributed over the entire 15 nucleobase sequence of the target oligonucleotide.

High Pressure Liquid Chromatography (HPLC), reverse-phase chromatography and ion-exchange chromatography are examples of commonly used traditional techniques for the purification of crude synthetic oligonucleotides (Warren et 20 al., Chapter 9 In: *Methods in Molecular Biology*, Vol. 26: *Protocols for Oligonucleotide Conjugates*, Agrawal, S., Ed., 1994, Humana Press Inc., Totowa, NJ, pages 233-264). However, due to the lack of chromatographic selectivity and product yield, the (n-1)-mer impurities are still present in 25 the full-length (i.e., n-mer) oligonucleotide product after the purification unless a very low yield of desired n-mer is acceptable. It is also known in the art to purify oligonucleotides by denaturing polyacrylamide gel electrophoresis (PAGE), but such methods are not applicable 30 to the mass production of oligonucleotides as the yields obtained by such methods are typically less than 50%. (Ausubel et al., eds., *Short Protocols in Molecular Biology*, 2nd Ed., Greene Publishing Associates and John Wiley & Sons, New York, 1992, p. 2-37). 35 The present invention provides new methods for the large-scale purification of oligonucleotide substances with superior selectivity and product yield, by solid-liquid

- 5 -

affinity extraction using immobilized affinity units that preferentially bind the desired full length oligonucleotide. The affinity unit comprises an immobilized nucleobase sequence having reverse complementarity to the desired 5 target oligonucleotide over a central portion thereof, the essentially full-length (p, as defined herein) thereof, or the full-length (n) thereof. The method of the invention allows for the purification of a desired full length synthetic oligonucleotide from a mixture of heterogeneous 10 failed sequence oligonucleotides.

Reese (Tetrahedron, 1978, 34, 3143) describes methods for synthesizing nucleoside building blocks for oligonucleotides and early attempts to achieve significant amounts of oligonucleotide synthesis using the 15 phosphotriester method.

U.S. Patent No. 4,458,066 to Caruthers et al. (issued July 3, 1984) describe a modification of the phosphite triester method for synthesizing oligonucleotides using nucleotide intermediates bound to inorganic polymer 20 supports.

Caruthers (Science, 1985, 230, 281) describes the phosphite triester method of oligonucleotide synthesis and at least partially automated machines for carrying out this method.

25 Uhlmann et al. (Chem. Reviews, 1990, 90, 543) review the then-prevailing state of the art of synthesis of unmodified and modified oligonucleotides.

Gilham (J. Am. Chem. Soc.; 1964, 86, 4982) describes the synthesis of oligo(dT) and its covalent 30 attachment to cellulose. A method of separating oligomers of adenylic acid using a column of this material is also described.

Seliger et al. (Tetrahedron Letts., 1978, 24, 2115) describe a method of oligonucleotide synthesis in 35 which the last residue incorporated into the desired n-mer is an affinity blocking group. Because the desired full-length (n) oligonucleotide includes the terminal affinity

- 6 -

blocking group, it can be separated from the undesired (i.e., (n-1), (n-2), etc.) products. In many instances, however, it is undesirable to retain the terminal affinity blocking group due to its effect on, e.g., biological 5 activity, and removing the terminal affinity group would be expected to decrease the yield of desired product.

Yashima et al. (*J. Chromatography*, 1992, 603, 111) describe the separation of nucleosides and nucleotide dimers via the use of affinity chromatography on silica gel columns 10 comprising immobilized nucleic acid analogs. Although oligonucleotides are also separable by the method of Yashima et al., it is noted that the resolving power of the system decreases as the target oligonucleotide length increases.

Temsamani et al. (*Nucl. Acids Res.*, 1995, 23, 1841) describe experiments in which the sequence identities of (n-1) [and some (n-2) and (n-3)] derivatives of a target full length oligonucleotide were determined. These experiments demonstrate the distribution of deleted nucleotides in failed sequences is greatest at the 3' 20 terminus of the target oligonucleotide.

International Publication No. WO 90/09393, published August 23, 1990, discloses a method of purifying full length target oligonucleotides from a mixture containing truncated sequences. The method, which depends 25 on hybridization between the 5' end of the target oligonucleotide and a short complementary oligonucleotide that is covalently bound to a solid support, does not distinguish between the target full length oligonucleotide and undesired oligonucleotides having a deletion of one or 30 more internal or 3' nucleotides (see also corresponding U.S. Patents No. 5,352,578 and No. 5,559,221, which issued October 4, 1994 and September 24, 1996, respectively).

International Publication No. WO 96/22299, published July 25, 1996, discloses a method of purifying 35 synthetic oligonucleotides from a sample containing the desired oligonucleotide and failed sequences. The disclosed method uses an anion exchange adsorbent that binds the

- 7 -

desired oligonucleotide in its protected form under conditions of high as well as low ionic strength. In some instances, additional ion exchange steps are required in order to separate the desired full length oligonucleotide 5 from shorter forms (page 6, lines 15-19).

To date, there is no known method for the large-scale purification of an oligonucleotide of length n that provides for the effective and cost-efficient separation of all forms of undesired deletion derivatives from the desired 10 full-length oligonucleotide. Consequently, there remains a need for compositions and methods that can be used to effectively purify a desired full length synthetic oligonucleotide from a heterogeneous mixture containing failed sequence oligonucleotides on a industrial scale in a 15 rapid and cost-effective manner.

SUMMARY OF THE INVENTION

In accordance with the present invention, methods and matrices are provided for the rapid, cost-effective and efficacious removal of undesired derivatives of a desired 20 target oligonucleotide. More particularly, the invention provides methods and matrices for the removal of all forms of undesired deletion derivatives [i.e., (n-1), (n-2), etc.] of the desired full length (n) oligonucleotide, including those undesired derivatives having an internal or 3' 25 deletion of one or more oligonucleotides. A desired level of large-scale purification of the desired oligonucleotide is achieved according to the methods and matrices of the invention. Such purification is achieved via the use of a matrix comprising an immobilized affinity unit. In one 30 embodiment, the affinity unit comprises a nucleobase sequence having a sequence that is the reverse complement of the target oligonucleotide over a central portion thereof, the essentially full-length (p, as defined herein) thereof, or the full-length (n) thereof. A mixture comprising the 35 desired target oligonucleotide is contacted with a matrix of the invention comprising the immobilized affinity unit under

- 8 -

stringent hybridization conditions, i.e., conditions under which the hybridization (binding) of the desired target oligonucleotide is preferentially achieved. The undesired [e.g., (n-1), (n-2), etc.] derivatives of the target 5 oligonucleotide hybridize poorly, or not at all, to the matrix of the invention under these conditions and are separated by relatively simple techniques (e.g., washing, centrifugation, etc.). Alternatively, the hybridization reaction occurs under conditions wherein some undesired 10 derivatives of the target oligonucleotide are initially bound but are subsequently removed by a first chemical or physical treatment (e.g., changes in pH, ionic strength, temperature, and the like) which result in a change in the environment of the mixture. Following separation of the 15 undesired derivatives of the target oligonucleotide, the bound target oligonucleotide is eluted from the affinity unit by application of a second chemical or physical treatment. The affinity unit remains immobilized to the matrix of the invention and, in a preferred embodiment, is 20 reused for purifying further batches of the desired target oligonucleotide.

These and other objects and features of the invention will become more fully apparent when the following detailed description of the invention is read in conjunction 25 with the accompanying figures and examples.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the modification of a primary amine, attached to a support (indicated by the open circle) by a linker (indicated by $(CH_2)_n$), by 1,4-phenylene 30 diisothiocyanate to form a modified support having a phenylisothiocyanate group.

Figure 2 shows the reaction of the primary amine group of a probe (i.e., affinity unit and spacer) with the isothiocyanate group of the modified support to form a 35 thiocarbamyl adduct, thereby covalently attaching the probe portion to the support via the linker.

- 9 -

Figure 3 shows an electropherogram of crude ISIS 2302.

Figure 4 shows an electropherogram of ISIS 2302 prepared by a method of the invention.

5 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The present invention is directed to methods for purifying a desired, full length oligonucleotide by the rapid, cost-effective and efficacious removal of contaminants, including but not limited to undesired deletion (e.g., n-1, n-2, etc.) derivatives of the desired (target) oligonucleotide. This is accomplished via solid-liquid extraction of the oligonucleotide through selective hybridization to an affinity unit that specifically binds the desired oligonucleotide. Matrices comprising such affinity units, useful for practicing the methods of the invention, are also herein provided. Although deletion derivatives of the desired target oligonucleotide are contaminants of particular concern, it will be appreciated by those skilled in the art that other contaminants can be removed by the method of the invention as well. For example, a significant portion of undesired salts can be removed using the method of the invention.

The target oligonucleotides that are purified by the methods and matrices of the invention include, in particular, those intended for uses requiring a relatively low concentration of undesired or uncharacterized contaminants. In particular, the invention is drawn to the purification of target oligonucleotides, particularly antisense oligonucleotides, intended for therapeutic delivery to an animal, including a human. Certain preferred embodiments of the present invention are drawn to the purification of target oligonucleotides designed to have therapeutic activity in an animal, such as a human; such target oligonucleotides may be formulated into a pharmaceutical composition. By "designed to have therapeutic activity," it is meant that the target

- 10 -

oligonucleotide is designed to function in a manner that is prophylactic, palliative or curative with regard to (1) a disorder caused by a hyperproliferation of cells (e.g., cancer), a pathogen (e.g., malaria, AIDS), or from causes 5 that appear to relate to neither pathogens nor hyperproliferative cells (e.g., Alzheimer's disease) or (2) the symptoms of such a disorder. The invention is also drawn to the purification of target oligonucleotides that modulate the expression of a cellular protein, including 10 cell surface proteins. In the context of this invention, "designed to modulate" means designed to either effect an increase (stimulate) or a decrease (inhibit) in the expression of a gene. Such modulation can be achieved by a variety of mechanisms known in the art, including but not 15 limited to transcriptional arrest; effects on RNA processing (capping, polyadenylation and splicing) and transportation; enhancement of cellular degradation of the target nucleic acid; and translational arrest (Crooke, S.T., et al., *Exp. Opin. Ther. Patents*, 1996, 6, 1). The following tables 20 list, as exemplars, some preferred target oligonucleotides that may be purified according to the methods and matrices of the invention, and the full-length embodiments of their corresponding affinity units. Such desired target oligonucleotides include, but are not limited to, those 25 designed to modulate the expression of cellular surface proteins (Table 1), and those designed to have therapeutic activity against disorders associated with cellular hyperproliferation (Table 2) or having no apparent pathological or hyperproliferative-related cause (Table 3) 30 and diseases resulting from eukaryotic pathogens (Table 4), retroviruses such as human immunodeficiency virus (HIV; Table 5) or viral pathogens other than retroviruses (Table 6).

- 11 -

TABLE 1: TARGET OLIGONUCLEOTIDES DESIGNED TO MODULATE CELL SURFACE PROTEINS AND AFFINITY UNITS THEREFOR

| 5 | Cell Surface Protein | Commercial/ Common Name (if any) | Target Oligonucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|---|----------------------|----------------------------------|---|---|
| | ICAM-1 | ISIS 2302 | 1 | 2 |
| | MDR | | 106,108,110,112 | 107,109,111,13 |

10 TABLE 2: TARGET OLIGONUCLEOTIDES DESIGNED TO HAVE THERAPEUTIC ACTIVITY AGAINST HYPERPROLIFERATIVE CELLS AND AFFINITY UNITS THEREFOR

| | Molecular Target | Commercial / Common Name (if any) | Target Oligonucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|----|---|-----------------------------------|---|---|
| 15 | c-myb | MYB-AS | 16 | 17 |
| | DNA methyl transferase | | 18,20 | 19,21 |
| | vascular endothelial growth factor (VEGF) | | 22,26,28,30,32,34,36,38,40,42 | 23,27,29,31,33,35,37,39,41,43 |
| | VEGF | Vm | 24 | 25 |
| | bcl-2 | | 44,46,48,50,52,54,56,58,60,62,64,66 | 45,47,49,51,53,55,57,59,61,63,65,67 |
| | bcl-2 | BCL-2 | 68 | 69 |
| | bcl-abl | | 76 | 77 |
| 20 | PKC-Z | | 70 | 71 |
| | PKC- α | ISIS 3521 | 72 | 73 |
| | c-raf | ISIS 5132 | 74 | 75 |

- 12 -

TABLE 3: TARGET OLIGONUCLEOTIDES DESIGNED TO HAVE
THERAPEUTIC ACTIVITY AGAINST NON-PATHOGENIC AND NON-
HYPERPROLIFERATIVE DISORDERS, AND AFFINITY UNITS THEREFOR

| Disorder | Commercial/ Common Name (if any) | Target Oligo- nucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|----------------------------|--|--|---|
| 5 Alzheimer's disease | | 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100 | 79, 81, 83, 85, 8 7, 89, 91, 93, 95 , 97, 99, 101 |
| Beta-thalassemia | 5'ss & 3'ss | 102 & 104 | 103 & 105 |

TABLE 4: TARGET OLIGONUCLEOTIDES DESIGNED TO HAVE
THERAPEUTIC ACTIVITY AGAINST EUKARYOTIC PATHOGENS AND
AFFINITY UNITS THEREFOR

| Pathogen / Disease | Commercial/ Common Name (if any) | Target Oligo- nucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|---|--|---|--|
| Plasmodium / malaria | | 114, 116, 118, 120 | 115, 117, 119, 1 21 |
| Schistosoma / bloodfluke infections | | 122 | 123 |

- 13 -

TABLE 5: TARGET OLIGONUCLEOTIDES DESIGNED TO HAVE
THERAPEUTIC ACTIVITY AGAINST RETROVIRUSES, INCLUDING HIV,
AND AFFINITY UNITS THEREFOR

| | Virus / Molecular Target | Commercial/ Common Name (if any) | Target Oligo-nucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|----|--------------------------------|----------------------------------|--|---|
| 5 | HTLV-III / primer binding site | | 124,126,128,130, 132,134 | 125,127,129,131, 133,135 |
| | HIV-1 / gag | GEM-91 | 136 | 137 |
| | HIV-1 / gag | GEM-92, GEM-93 | 138,140,142,144,146, 148,150,152,154 | 139,141,143,145,147,149,151, 153,155 |
| 10 | HIV | AR 177 | 156 | 157 |
| | HIV / tat, vpr, rev, env, nef | | 158,160,162 | 159,161,163 |
| | HIV / pol, env, vir | | 164,166,168,170, 172,174,176,178 | 165,167,169,171, 173,175,177,179 |
| 15 | HIV-1 / tat, rev, env, nef | | 180,181;183,184; 186;188 | 182;185;187;189 |
| | HIV / gp120 | ISIS 5320 | 190 | 191 |

- 14 -

TABLE 6: TARGET OLIGONUCLEOTIDES DESIGNED TO HAVE
THERAPEUTIC ACTIVITY AGAINST NON-RETROVIRAL VIRUSES AND
AFFINITY UNITS THEREFOR

| | Virus / Molecular Target | Commercial/ Common Name (if any) | Target Oligo-nucleotide Seq. SEQ ID NO(S): | Affinity Unit Nucleobase Seq. SEQ ID NO(S): |
|----|-----------------------------|----------------------------------|--|--|
| 5 | influenza virus | | 192, 194, 196, 198, 200, 202, 204, 206, 208 | 193, 195, 197, 19 9, 201, 203, 205, 207, 20 9 |
| 10 | Epstein-Barr Virus | | 228, 230, 232 | 229, 231, 233 |
| | Respiratory Syncytial Virus | | 234, 236, 238, 240 | 235, 237, 239, 24 1 |
| | cytomegalovirus (CMV) | | 212, 214, 216, 218, 220, 222, 224, 226 | 213, 215, 217, 21 9, 221, 223, 225, 22 7 |
| 15 | CMV | GEM-132 | 210 | 211 |
| | CMV | ISIS 2922 | 242 | 243 |

15 Target oligonucleotides that may be purified according to the methods and matrices of the invention include those consisting of naturally occurring nucleotides as well as those comprising one or more chemical modifications. Specific examples of some modified
20 oligonucleotides that can be incorporated into the target oligonucleotide include those containing phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl, cycloalkyl, heteroatomic or heterocyclic intersugar linkages. Specifically, such oligonucleotides include those
25 having phosphorothioates intersugar linkages, those with heteroatomic intersugar linkages including $\text{CH}_2-\text{NH}-\text{O}-\text{CH}_2$, $\text{CH}_2-\text{N}(\text{CH}_3)-\text{O}-\text{CH}_2$ [known as a methylene(methylimino) or MMI backbone], $\text{CH}_2-\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2$, $\text{CH}_2-\text{N}(\text{CH}_3)-\text{N}(\text{CH}_3)-\text{CH}_2$ and $\text{O}-\text{N}(\text{CH}_3)-\text{CH}_2-\text{CH}_2$ backbones, wherein the native phosphodiester backbone

is represented as O-P(=O)-CH₂), those with heterocyclic linkages including the morpholino sugar-backbone structures (Summerton and Weller, U.S. No. Patent 5,034,506) or those with a peptide nucleic acid (PNA) backbone (in which the 5 phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone wherein the nucleobases is bound directly or indirectly to an aza nitrogen atoms of the polyamide backbone (Nielsen et al., *Science*, 1991, 254, 1497). Specific examples of modified oligonucleotides also 10 include oligonucleotides containing one or more substituted sugar moieties [i.e., sugar moieties comprising one of the following at the 2' position: -F; -Cl; -Br; -OH; -SH; -SCH₃; -OCN; -OCH₂OCH₃; -O(CH₂)_nO(CH₂)_mCH₃, (i.e., alkoxyalkoxy), -O(CH₂)_nNH₂ or -O(CH₂)_nCH₃ where m is from 0 to about 6 and n is 15 from 1 to about 10; C₁ to C₁₀ lower alkyl, substituted lower alkyl, alkaryl or aralkyl; -CN; -CF₃; -OCF₃; O-, S-, or N-alkyl or substituted alkyl; O-, S-, or N-alkenyl; -SOCH₃; -SO₂CH₃; -ONO₂; -NO₂; -N₃; -NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; 20 substituted silyl; an RNA cleaving group; a reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an oligonucleotide and other substituents having similar properties]. Particularly 25 preferred embodiments include 2'-alkoxyalkoxy substituents such as 2'-O-methoxyethoxy [2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl)] (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486), 2'-methoxy (2'-O-CH₃), 2'-propoxy (2'-OCH₂CH₂CH₃) and 2'-fluoro (2'-F). Additional modified oligonucleotides 30 include those having similar modifications at other positions on the oligonucleotide (particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide), oligonucleotides having sugar mimetics such as cyclobutyls in place of the 35 pentofuranosyl group or base modifications or substitutions

- 16 -

(e.g., with a "universal" base such as inosine).

A further modification of the target oligonucleotide involves chemically linking to the target oligonucleotide one or more lipophilic moieties which

5 enhance the cellular uptake of the oligonucleotide. Such lipophilic moieties include but are not limited to a cholesteryl moiety (Letsinger et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1989, 86, 6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g.,

10 hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al.,

15 *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Letts.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36,

20 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantane acetic acid (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923). Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides, are disclosed in U.S. Patents No.

25 30 5,138,045, No. 5,218,105 and No. 5,459,255.

The target oligonucleotide can also be an oligonucleotide which is a chimeric oligonucleotide including a "gapmer" or a "hemimer." Chimeric oligonucleotides are oligonucleotides which contain two or

- 17 -

more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one terminal region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased 5 resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the intracellular target nucleic acid. In a "hemimer," a single terminal (either 5' or 3') region is so modified in the oligonucleotide structure. When both termini of the 10 oligonucleotide are modified, the oligonucleotide is called a Agapmer® and the modified 5'- and 3'-terminal regions are referred to as "wings"; an additional, typically central, region (typically referred to as the "gap" or "core") of the oligonucleotide may serve as a substrate for cellular 15 enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. In a gapmer, the 5' and 3' wings can be modified in the same or different manner depending on what properties it is desired to achieve.

The length of a target oligonucleotide is defined 20 as "n" nucleobases, wherein "n" is a positive whole number. The length of target oligonucleotides that can be purified according to the methods and matrices of the invention is one having from 5 to about 60 nucleobases, preferably from 7 to about 50 nucleobases, more preferably from 8 to about 40 25 nucleobases, even more preferably from 9 to about 30 nucleobases and most preferably from 10 to about 25 nucleobases. The method of synthesis of the target oligonucleotide does not typically effect the methods of the invention; in general, any method of synthesis, including 30 methods for the solution phase synthesis of oligonucleotides (see U.S. Patent No. 5,210,264 to Yau, assigned to the present applicants; Reese et al., *J. Chem. Soc. Perkin Trans.*, 1993, 1, 2291; and Wada et al., *Tetrahedron*, 1993, 49, 2043) may be used.

35 The matrices of the invention comprise a plurality of a molecular composition that comprises several parts: (1)

- 18 -

a support, (2) an optional linker to the support, (3) an optional spacer and (4) a unit having a high degree of specific affinity to the target oligonucleotide (affinity unit). When present, the linker (2) and spacer (3) provide 5 a bridge between the support (1) and the affinity unit (4) in such a way as to not significantly alter or reduce the binding capacity of the latter element. The probe of the matrix of the invention comprises parts (4) and, optionally, (3). The following paragraphs describe the matrices of the 10 invention in more detail.

With regard to the support (1), a variety of substances may be used. A suitable support has preferred characteristics of non-reactivity with compounds introduced during the various steps of oligonucleotide synthesis, 15 accessibility to solvents utilized during such syntheses, and a tendency towards minimal barrier layer diffusion. A barrier layer is created by an ordering of solvent molecules on the surface of a solid phase support. As this barrier layer is composed of ordered molecules, it is difficult to 20 get consistent reagent diffusion across such a barrier to the molecules of interest which are attached to the support. It will be appreciated by those skilled in the art that the chemical composition of the affinity unit (4), the optional linker (2) and/or the optional spacer (3) may influence the 25 choice of the support (1). The support may be insoluble ("solid") or soluble. The chemical nature of various solid supports, and their desirable properties, are reviewed by Winter (Chapter 17 *In: Combinatorial Peptide and Nonpeptide Libraries: A Handbook*, Jung, ed., VCH Publishers, Inc., New 30 York, NY, 1996, pp. 465-510). Suitable solid supports include, but are not limited to, graft polymers (U.S. Patent No. 4,908,405 to Bayer and Rapp); polyacrylamide (Fahy et al., *Nucl. Acids Res.*, 1993, 21, 1819); polyacrylmorpholide, polystyrene and derivatized polystyrene resins (Syvanen et 35 al., *Nucl. Acids Res.*, 1988, 16, 11327; U.S. Patent Nos. 4,373,071 and 4,401,796 to Itakura), including amino methyl styrene resins (U.S. Patent No. 4,507,433 to Miller and

- 19 -

Ts'0); copolymers of N-vinylpyrrolidone and vinylacetate (Seliger et al., *Tetrahedron Letts.*, 1973, 31, 2911; Seliger et al., *Die Makromolekulare Chemie*, 1975, 176, 609; and Seliger, *Die Makromolekulare Chemie*, 1975, 176, 1611);

5 TEFLON™ (Lohrmann et al., *DNA*, 1984, 3, 122; Duncan et al., *Anal. Biochem.*, 1988, 169, 104); controlled pore glass (Chow et al., *Anal. Biochem.*, 1988, 175, 63); polysaccharide supports such as agarose (Kadonaga, *Methods Enzymol.*, 1991, 208, 10; Arndt-Jovin et al., *Eur. J. Biochem.*, 1975, 54, 10 411; Wu et al., *Science*, 1987, 238, 1247; Blank et al., *Nucleic Acids Res.*, 1988, 16, 10283) or cellulose (Goldkorn et al., *Nucl. Acids Res.*, 1986, 14, 9171; Alberts et al., *Meth. Enzymol.*, 1971, 21, 198) or derivatives thereof, e.g., DEAE-cellulose (Schott, *J. Chromatogr.*, 1975, 115, 461) or

15 phosphocellulose (Siddell, *Eur. J. Biochem.*, 1978, 92, 621; Bunemann et al., *Nucl. Acids Res.*, 1982, 10, 7163; Noyes et al., *Cell*, 1975, 5, 301; Bunemann et al., *Nucl. Acids Res.*, 1982, 10, 7181); dextran sulfate (Gingeras et al., *Nucl. Acids Res.*, 1987, 15, 5373); polypropylene (Matson et al., *Anal. Biochem.*, 1994, 217, 306); agarose beads (Kadonaga et al., *Proc. Natl. Acad. Sci. U.S.A.*, 1986, 83, 5889); latex particles (Kawaguchi et al., *Nucleic Acids Res.*, 1989, 17, 6229); nylon beads (Van Ness et al., *Nucl. Acids Res.*, 1991, 19, 3345); paramagnetic beads (Gabrielson et al., *Nucl. Acids Res.*, 1989, 17, 6253; Day et al., *Biochem. J.*, 1991, 278, 735); silica gels (Yashima et al., *J. Chromatogr.*, 1992, 603, 111); derivatized forms of silica gels, polytetrafluoroethylene, cellulose or metallic oxides (U.S. Patent No. 4,812,512 to Buendia); and art-recognized

20 equivalents of any of the preceding solid supports. In one set of preferred embodiments, the solid support is a crosslinked copolymer of N-vinylpyrrolidone, other N-vinyl-lactam monomers and an ethylenically unsaturated monomer having at least one amine or amine-displaceable functionality

25 as disclosed in U.S. Patent No. 5,391,667. In another set of preferred embodiments, polystyrene or long chain alkyl CPG (controlled pore glass) beads are employed as the solid

- 20 -

support. In a further set of preferred embodiments, the support is soluble and is composed of, for example, modified polyethylene glycol (PEG) units (Bonora et al., *Nucleic Acids Res.*, 1993, 21, 1213; Bagno et al., *Chem. Biochem. Eng. Q.*, 5 1994, 8, 183); PEG-based matrices having the advantage of being reversibly precipitable from solution.

With regard to the optional linker (2), a variety of chemical linking groups or chains may be employed in the matrix of the invention. Any chemical group or chain 10 capable of forming a stable chemical linkage, or a stable association, between the support (1) and the affinity unit (4), or between the support (1) and the optional spacer (3), may be employed. A suitable linker has preferred characteristic of non-reactivity with compounds introduced 15 during the various steps of oligonucleotide synthesis. It will be appreciated by those skilled in the art that the chemical composition of the support (1) and the affinity unit (4) and/or the optional spacer (3) may influence the choice of the linker. Many suitable linkers will comprise a 20 primary amine group at either or both termini, as many chemical reactions are known in the art for linking primary amine groups to a variety of other chemical groups. However, other terminal reactive moieties are known and may be used in the invention. Suitable linkers include, but are 25 not limited to, linkers having a terminal thiol group for introducing a disulfide linkages to the support (Day et al., *Biochem. J.*, 1991, 278, 735; Zuckermann et al., *Nucl. Acids Res.*, 15, 5305); linkers having a terminal bromoacetyl group for introducing a thiol-bromoacetyl linkage to the support 30 (Fahy et al., *Nucl. Acids Res.*, 1993, 21, 1819); linkers having a terminal amino group which can be reacted with an activated 5' phosphate of an oligonucleotide (Takeda et al., *Tetrahedron Letts.*, 1983, 24, 245; Smith et al., *Nucl. Acids Res.*, 1985, 13, 2399; Zarytova et al., *Anal. Biochem.*, 1990, 35 188, 214); poly(ethyleneimine) (Van Ness et al., *Nucl. Acids Res.*, 1991, 19, 3345); acyl chains (Akashi et al., *Chem. Lett.*, 1988, 1093; Yashima et al., *J. Chromatogr.*, 1992,

- 21 -

603, 111); polyvinyl alcohol (Schott, *J. Chromatogr.*, 1975, 115, 461); alkyl chains (Goss et al., *J. Chromatogr.*, 1990, 508, 279); alkylamine chains (Pon et al., *BioTechniques*, 1988, 6, 768); biotin-avidin or biotin-streptavidin linkages 5 (Kasher et al., *Mol. Cell. Biol.*, 1986, 6, 3117; Chodosh et al., *Mol. Cell. Biol.*, 1986, 6, 4723; Fishell et al., *Methods Enzymol.*, 1990, 184, 328); and art-recognized equivalents of any of the preceding linkers. In a preferred embodiment of the invention, an *n*-aminoalkyl chain is the 10 linker. In a particularly preferred embodiment of the invention, in which an oligonucleotide chain constitutes both the spacer (3) and the affinity unit, an *n*-aminohexyl chain [i.e., NH₂-(CH₂)₅] is the linker (2).

With regard to the optional spacer (3), a variety 15 of chemical groups or chains may be employed in the matrix of the invention. Any chemical group or chain capable of forming a stable chemical linkage, or a stable association, between the support (1) and the affinity unit (4), or between the support (1) and the optional linker (2), may be 20 employed. A suitable spacer has preferred characteristic of non-reactivity with compounds introduced during the various steps of oligonucleotide synthesis. It will be appreciated by those skilled in the art that the chemical composition of the support (1) and the affinity unit (4) and/or the 25 optional linker (2) may influence the choice of the spacer. Typically suitable spacers include, but are not limited to, oligopeptides; oligonucleotides; alkyl chains; polyamines; polyethylene glycols; oligosaccharides; and art-recognized equivalents of any of the preceding spacers. In one set of 30 preferred embodiments of the invention, the spacer is an alkyl chain, most preferably a C₁-C₂₀ alkyl chain. In another set of preferred embodiments of the invention, the spacer is an oligonucleotide chain, particularly an oligonucleotide chain that comprises one or more chemical modifications that 35 render it resistant to chemical attack. In this set of preferred embodiments, an oligodeoxyribonucleotide chain is particularly preferred. In a particularly preferred

- 22 -

embodiment of the invention, oligo(dT)₅₋₃₀ acts as the spacer of the matrix of the invention. This preferred spacer has the following advantages. This spacer is composed of nucleotides and is thus closely related in chemical 5 properties to a preferred affinity unit, i.e., an oligonucleotide. This chemical relatedness provides the benefit of placing the affinity unit in a context that is likely to be appropriate for nucleic acid hybridization duplexing. Although other oligonucleotides [e.g., 10 oligo(dA), oligo(dG) or oligo (dC)] might be employed for the spacer, a preferred spacer is more chemically stable.

It will be appreciated by those skilled in the art that the optional linker (2) and the optional spacer (3) can be combined into one unit. Furthermore, the linker and 15 spacers need not comprise distinct chemical groups or chains. For example, an appropriate oligopeptide or oligonucleotide chain could function as a combined linker and spacer of the matrix of the invention. Thus, suitable linker/spacers include, but are not limited, to the linker 20 and spacers described above. Methods of determining an appropriate (i.e., providing the optimal degree and specificity of hybridization between the affinity unit and the target oligonucleotide) length of linker/spacers are known in the art (see, for example, Day et al., *Biochem. J.*, 25 1991, 278, 735). In general, however, for oligonucleotide synthetic conditions that include basic conditions, the linker (2) or spacer (3) of the matrix of the invention would not include carbonate groups. The carbonate moiety is relatively unstable to basic reagents used in some 30 oligonucleotide syntheses and to contaminants (mainly bases) that may be found in solvents utilized in such oligonucleotide synthesis.

With regard to the affinity unit (4), this portion of the matrix of the invention has the characteristic of 35 binding specifically (or at least preferentially) yet reversibly to a portion of a target oligonucleotide, the purification of the target oligonucleotide being the object

- 23 -

of the invention. The portion of the target oligonucleotide that is specifically bound by the affinity unit is referred to as its "hybridizing portion" herein. A preferred affinity unit is one that comprises a chemical composition 5 having a nucleobase sequence that is the reverse complement of the hybridizing portion of the nucleobase sequence of the target oligonucleotide. The hybridizing portion may be a central portion, a terminal portion, or the majority or even entirety of the nucleobase sequence of the target 10 oligonucleotide. The term "a central portion" is intended to encompass preferably from at least five, or from at least ten, contiguous nucleobases derived from the section of the target oligonucleotide's sequence that is internal from the target oligonucleotide's 3' and 5' "terminal portions." A 15 terminal portion includes the most 3' or 5' nucleobase of a target oligonucleotide and comprises an additional number, r , of immediately contiguous nucleobases of the target oligonucleotide, wherein r is from 2 to about 10 nucleobases. An affinity unit having a nucleobase sequence 20 that is the reverse complement of a central portion of the nucleobase sequence of the target oligonucleotide will hybridize with high affinity to the target oligonucleotide, but not to, e.g., deletion derivatives lacking one or more nucleobases in the central portion. In another embodiment, 25 the nucleobase sequence of the affinity unit is "full-length", i.e., the same length (n) of the target oligonucleotide, and which is the reverse complement of that of the target oligonucleotide. Alternatively, the nucleobase sequence of the affinity oligonucleotide can be 30 "essentially full-length", i.e., having a length, p , wherein p is a positive whole number ranging from 4 to $n+4$, wherein, in a duplex between the target oligonucleotide and the nucleobase sequence of the affinity oligonucleotide, neither the 5' overhang nor the 3' overhang of said target 35 oligonucleotide is greater than two nucleobases, provided that, over length p , the nucleobase sequence of the affinity oligonucleotide is the reverse complement of the nucleotide

- 24 -

sequence of the target oligonucleotide. The nucleobase sequence of the affinity unit can be from 5 to 60 nucleobases in length, preferably from 10 to 40 nucleobases in length, more preferably from 11 to 30 nucleobases in 5 length and most preferably from 12 to 25 nucleobases in length. Affinity units of differing chemical compositions (e.g., oligodeoxynucleotides, oligoribonucleotides and peptide nucleic acids) can be employed in the invention, although certain compositions may be preferred in particular 10 instances. For example, when nucleases are suspected of being present in the initial mixture from which the target oligonucleotide is to be purified, or when nucleases from biological contaminants might eventually be present in a matrix that is stored and reused, compositions that are 15 relatively nuclease resistant might be preferred. Such relatively nuclease resistant compositions include, for example, oligodeoxyribonucleotides and peptide nucleic acids. Unlike RNA nucleases, for which no "universal" inhibitor is known, all characterized DNA nucleases require 20 a divalent metal ion for catalysis and are thus inhibited by chelating agents such as EDTA (Jarrett, *J. Chromatogr.*, 1993, 618, 315); oligodeoxyribonucleotides can thus be more simply prevented from nuclease degradation than oligoribonucleotides. Peptide nucleic acids, which are not 25 degraded by either nucleases or proteases, exhibit particularly stringent specificities for their complementary oligonucleotides, and may thus provide the best separation from undesired derivative oligonucleotides in some instances.

30 The method of synthesis of the affinity unit does not typically effect the methods of the invention; in general, any method of synthesis of the particular sort of affinity unit, including methods for the solution phase synthesis of oligonucleotides (see U.S. Patent No. 5,210,264 35 to Yau, assigned to the present applicants; Reese et al., *J. Chem. Soc. Perkin Trans.*, 1993, 1, 2291; and Wada et al., *Tetrahedron*, 1993, 49, 2043) may be used. After its

- 25 -

synthesis, the affinity unit (or, if both the spacer portion and affinity unit are oligonucleotidic) is purified by HPLC (high pressure liquid chromatography). Because the presence of deletion derivatives in the affinity unit may result in 5 the undesired binding and retention of deletion derivatives of the target oligonucleotide, it may be necessary to purify the affinity unit in a manner that achieves significant purity thereof at the expense of a reduced yield of the affinity unit. On the other hand, certain embodiments of 10 the invention are more tolerant of impurities in the affinity unit than others. For example, purification of oligonucleotides via multiple rounds of affinity chromatography, wherein a different affinity unit is used during each round of purification (see Example 8), exposes 15 the target oligonucleotide to two or more "screens" (matrices) with one likely result being a further removal of contaminating impurities from the target oligonucleotide mixture.

By stating that the oligonucleotide of the 20 affinity unit has a sequence that is the "reverse complement" of that of the nucleotide sequence, the following features are intended. As is known in the art, a nucleic acid duplex is formed of two antiparallel strands, i.e., strands that hybridize to each other in a "head-to- 25 tail" fashion:

Strand 1: 5' -----> 3'
Strand 2: 3' <----- 5'

Specific nucleobases in the interior of a nucleic acid duplex bind to specific partner nucleobases. Among the 30 naturally occurring nucleobases, guanine (G) binds to cytosine (C), and adenine (A) binds to thymine (T) or uracil (U). Thus, in the above diagram, Strand 2 will have a nucleotide sequence that is the reverse complement of Strand 1, i.e., Strand 2 will have, in "reverse" (3' to 5') order, 35 the partner ("complement") nucleobases to those of Strand 1.

- 26 -

The sequence of the oligonucleotide of the affinity unit can have reverse complementarity to the target oligonucleotide through a variety of equivalents. In addition to the equivalency of U (RNA) and T (DNA) as partners for A, other 5 naturally occurring nucleobase equivalents are known, including 5-methylcytosine, 5-hydroxymethylcytosine (HMC), glycosyl HMC, gentiobiosyl HMC (C equivalents), and 5-hydroxymethyluracil (U equivalent). Furthermore, synthetic nucleobases which retain partner specificity are known in 10 the art and include, for example, 7-deaza-guanine, which retains specificity for C. Thus, reverse complementarity will not be altered by any chemical modification to a nucleobase in the nucleotide sequence of the affinity oligonucleotide which does not alter its specificity for the 15 partner nucleobase in the target oligonucleotide. Moreover, in instances when the target oligonucleotide comprises a mixture of nucleobases at one or more positions within its sequence, reverse complementarity can be achieved by inserting a "universal" base partner, e.g., hypoxanthine 20 (inosine, I, is the corresponding nucleotide) at the corresponding position in the affinity unit. By way of example, an affinity unit having an affinity oligonucleotide having the nucleotide sequence 5'-GGGICGCG has a sequence that is the reverse complement of the target oligonucleotide 25 mixture [5'-CGCGACCC, 5'-CGCGGCCC, 5'-CGCGTCCC and 5'-CGCGCCCC].

In embodiments wherein the nucleobase sequence of the affinity unit is essentially full-length, the duplex between the nucleobase sequence of the affinity unit and the 30 hybridizing portion of the target oligonucleotide will result in the target oligonucleotide having either a "3' overhang" or a "5' overhang" or, in some instances, both types of overhangs. A "3' overhang" consists of unpaired nucleotides on the 3' terminus of the target 35 oligonucleotide, whereas a "5' overhang" consists of unpaired nucleotides on the 5' terminus of the target oligonucleotide. The two types of overhangs may be

- 27 -

diagramed as follows:

3' overhang of target oligonucleotide:

5' overhang of target oligonucleotide:

10 Target (length n): 5' -overhang- 3'
 Affinity unit (length p): 3' <----- 5'

It will be appreciated by those skilled in the art that a preferred affinity unit will selectively bind and/or retain the target oligonucleotide with high affinity and specificity under conditions wherein derivatives of said target oligonucleotide having one or more mismatches with the nucleobase sequence of said affinity unit are not bound and/or retained. In various embodiments of the invention, the affinity unit can incorporate one or more chemical modifications for the purpose of enhancing specific interactions with the target oligonucleotide, as is described in more detail in Example 6. Such modifications may additionally or alternatively result in the affinity unit having increased resistance to degradative contaminants, e.g., exonucleases. The target oligonucleotide may additionally or alternatively comprise such modifications, so long as reverse complementarity is maintained between the target oligonucleotide and that of the affinity unit over the above-defined "hybridizing" portion of the target oligonucleotide or lengths "n" (full-length) or "p" (essentially full-length) thereof.

It will be appreciated by those skilled in the art that the optional linker (2), optional spacer (3) and 35 affinity unit (4) can be combined into one unit.

Furthermore, the linker, spacer and affinity unit need not comprise distinct chemical groups or chains. For example, an appropriate oligonucleotide chain could function as the linker, spacer and affinity unit of the matrix of the 5 invention.

It will be further appreciated by those skilled in the art that the probe of the matrix of the invention, comprising affinity unit (4) and, optionally, the spacer (3), can be combined into one unit. Furthermore, the spacer 10 and affinity unit need not comprise distinct chemical groups or chains. Thus, in a preferred embodiment, an aminohexyl group is the linker (2) to the support (1), as it is easily attached to the 5' end of a oligonucleotide by a solid phase synthesizer. In this embodiment, which is described in more 15 detail in the Examples, the probe includes an oligonucleotide which comprises a first nucleotide sequence, which functions as the spacer (3), and a second nucleotide sequence, which serves as the affinity unit (4).

It will also be appreciated by those skilled in 20 the art that the affinity unit (4) can be attached to the spacer (3), linker (2) or support (1) at any position thereof so long as the potential for hybridization with the target oligonucleotide is not negatively effected. That is, the affinity unit can be attached to the spacer, linker or 25 support at its 3' or 5' terminus, or through a position on its backbone or one or more of its sugar residues or nucleobases, so long as the portion of the affinity unit that hybridizes to the hybridizing portion of the target oligonucleotide remains accessible for binding.

30 In one embodiment, the affinity unit (4) is synthesized directly on the support (i.e., *in situ*) rather than being separately synthesized and subsequently attached to the support. This embodiment is particularly useful when the components of the affinity unit, and the components 35 linking it to the support, are stable under the various conditions of synthesis and subsequent chemical steps (deprotection, deblocking and the like) necessary to prepare

- 29 -

the matrix for use in the method of the invention. Examples of *in situ* synthesis of oligonucleotides on both soluble and insoluble supports are known in the art (Bonora et al., *Nucleic Acids Res.*, 1993, 21, 1213; Bagno et al., *Chem. 5 Biochem. Eng. Q.*, 1994, 8, 183; Matson et al., *Anal. Biochem.*, 1994, 217, 306; Maskos et al., *Nucl. Acids Res.*, 1992, 20, 1679; Southern et al., *Genomics*, 1992, 13, 1008; Sheldon et al., in: *Matrix DNA Hybridization, Nucleic Acid Conference*, San Diego, 1992; Cashion et al., *Nucl. Acids 10 Res.*, 1977, 4, 2593; Duncan et al., *Anal. Biochem.*, 1988, 169, 104).

The method of the invention comprises at least 2 steps: (a) contacting a mixture comprising the target oligonucleotide and undesired deletion sequence 15 oligonucleotides to the matrix of the invention under conditions such that a hybridization reaction preferentially occurs between the target oligonucleotide and the affinity unit, and (c) dissociating and recovering the target oligonucleotide from the matrix of the invention.

20 Optionally, the method of the invention additionally comprises step (b), removing unbound, undesired deletion sequence oligonucleotides or other undesirable contaminants from the matrix by, for example, washing the matrix while the target oligonucleotide is bound thereto.

25 Step (a) is a hybridization step in which a mixture of crude synthetic oligonucleotides, comprising the desired full length n-mer as well as undesired derivatives [i.e., (n-1), (n-2), etc.] is contacted to the matrix of the invention and allowed to hybridize to the affinity unit.

30 The degree of hybridization between the affinity unit and the full length (n) target oligonucleotide is dependent upon parameters such as the ionic strength of the buffer solution in which the hybridization occurs, temperature, base composition and length of the duplex formed between the 35 target oligonucleotide and the affinity unit, concentration of the affinity unit, concentration of the target oligonucleotide, and the concentration(s) of duplex

- 30 -

destabilizing agent(s). The method of the invention is designed to maximize the affinity of the affinity unit for the full length target oligonucleotide while achieving the least degree of affinity for undesired (deletion sequence) 5 oligonucleotides.

The following serve as examples of the buffer solution can be applied:

10 and
(1) SSPE buffer (1x-5x); 5x SSPE buffer is 0.75 M NaCl, 50 mM Na₂PO₄, pH 7.4, and 5 mM EDTA;

(2) 1 M KOAc or NaOAc buffer, pH 6.5.

15 As will be appreciated to those skilled in the art, it is preferable to remove nucleases and duplex destabilizing agents from such buffers before their use in hybridization reactions. This can be achieved by, for example, autoclaving the buffers.

Temperature can be another important parameter for hybridization reactions. In the method of the invention. In certain preferred embodiments of the invention, the 20 temperature of the hybridization reaction is adjusted so that only full length target oligonucleotide will quantitatively hybridize to the affinity unit. At optimum temperatures, the formation of duplexes between the affinity unit and undesired oligonucleotides (deletion sequences) 25 will be thermodynamically disfavored. Although, as is known in the art, optimum temperatures can be estimated for various chromatographic methods involving nucleic acids, some degree of "fine tuning" will be required in many instances. For example, Jarrett (J. Chromatogr., 1993, 618, 30 315) opines that the equation given by Sambrook et al. (Molecular Cloning-A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2nd Ed., 1989, pages 9.47-9.51) is "the most useful" in chromatography experiments, but notes that "[s]ome cautions must apply to 35 the use of these equations."

The methods of the invention optionally comprise the step (b), removing undesired oligonucleotides and/or other undesired contaminants, which may be carried out, for example, by placing the hybridized matrix:target

5 oligonucleotide complexes into a suitable washing buffer which has a composition that is similar, or even identical, to that of the hybridization buffer of step (a) but which is different in concentration. When identical in composition to the hybridization buffer, the washing buffer can be from

10 0.4x to 2x, preferably from 0.5x to 1.6x, and most preferably from 0.6x to 1.2x the concentration of the hybridization buffer. By way of example, if the hybridization buffer is 3x SSPE buffer, the washing buffer is from 1.2x to 6x SSPE buffer, preferably from 1.5x to 4.8x

15 SSPE buffer, and most preferably from 1.8x to 3.6x SSPE buffer. In an alternative embodiment, the temperature at which the hybridization reaction occurs is increased so that undesired oligonucleotides do not hybridize as well to the affinity unit. In general, the temperature for

20 hybridization reactions should be between the melting temperature of the full length oligonucleotide duplex, T_m^u , and the highest melting temperature of deletion sequence oligonucleotides, T_m^d . That is, the temperature range at which the hybridization reaction is performed, T, is defined

25 by the equation

$$T_m^d < T < T_m^u.$$

Methods for estimating and determining these parameters are known in the art (see, for example, Lehninger, *Biochemistry*, 2nd Ed., 1970, Worth Publishers Inc., New York, NY, page 875; Jarrett, *J. Chromatogr.*, 1993, 618, 315; Freier, Chapter 5 In: *Antisense Research and Applications*, Crooke et al., Eds., 1993, CRC Press, Boca Raton, LA, pp. 67-82). The purpose of optional step (b) is to remove as many deletion sequence oligonucleotide molecules and other undesirable

30 35 contaminating molecules as possible while maintaining the

- 32 -

highest possible concentration of hybridized full length oligonucleotide bound to the affinity unit of the support. Accordingly, the specific conditions at which these steps are carried out may be adjusted by monitoring these 5 parameters and others known to those skilled in the art.

Step (c), dissociation and recovery of the full length target oligonucleotide, is achieved, for example, by placing the matrix:target oligonucleotide complexes into distilled water. The target oligonucleotide is thus easily 10 and readily dissociated from the matrix of the invention and is recovered in the distilled water. Temperature and time are two parameters that can be adjusted to achieve optimal elution. In a preferred embodiment, the full length oligonucleotide is dissociated and recovered (1) in 15 distilled water, ensuring a low salt content in the final product and thus eliminating an expensive desalting step, and (2) at a temperature higher than that at which the hybridization step (a) occurred in order to facilitate the complete release of the target oligonucleotide. This 20 preferred embodiment eliminates the need for an expensive and time-consuming desalting step that is present in many other methods of oligonucleotide purification. If desired, the presence of oligonucleotides in the flow-through during the recovery step (c), or any other step(s), can be 25 monitored by a variety of methods known in the art (see, for example, Jarrett, *J. Chromatogr.*, 1993, 618, 315).

In a preferred embodiment of the invention, the methods and matrices of the invention are used to purify oligonucleotides prepared by a synthesis procedure that is 30 "blockwise," i.e., one in which one or more coupling steps results in the incorporation of a "blockmer" of nucleobase units (e.g., a dinucleotide or trinucleotide). As used herein, a "blockmer" refers to a chemically linked sequence of 2, 3, 4, 5, 6, 7 or 8 nucleobases that is incorporated 35 into an oligonucleotide *en toto*. Oligonucleotide synthesis procedures that utilize at least one step in which several nucleobases are incorporated in one step are known in the

art (see, e.g., Kumar et al., *J. Org. Chem.*, 1984, 49, 4905; Bannwarth, *Helv. Chimica Acta*, 1985, 68, 1907; Cosstick et al.; *Biochemistry*, 1985, 24, 3630; Wolter et al., *Nucleosides & Nucleotides*, 1986, 5, 65; Miura et al., *Chem. Pharm. Bull.*, 1987, 35, 833; Yau et al., *Tetrahedron Letts.*, 1990, 31, 1953).

The purification, by the methods and materials disclosed herein, of target oligonucleotides prepared by blockwise synthesis procedures (in contrast to synthesis procedures which incorporate a single nucleobase per coupling step) is a preferred embodiment of the invention for the following reasons. A synthesis procedure that couples, for example, dinucleotides rather than mononucleotides results in a final reaction mixture wherein the contaminating undesired products are, for the most part, (n-2), (n-4), (n-6), etc. in length relative to the desired n-mer target oligonucleotide. Like all final reaction mixtures resulting from blockwise synthesis procedures, an [(n-2), (n-4), (n-6), etc.] mixture has the inherent advantage over final reaction mixtures comprising (n-1) derivatives, because some (n-1) derivatives can be particularly difficult to separate from the desired n-mer. The method of purification of a desired target oligonucleotide of the invention, which is effective in selectively removing single base (n-1) deletions from a mixture, is expected to be particularly effective when the method is applied to a "blockwise" final reaction mixture that has fewer undesired derivative oligonucleotides of the (n-1) type, albeit containing undesired derivative oligonucleotides having deletions greater than one nucleobase [i.e., (n-2), (n-3), (n-4), (n-5) or (n-6) bases]. Furthermore, it is possible in some instances to "fix" a desired chemical feature (e.g., a stereospecific chemical linkage) in a blockmer unit that would otherwise be randomized by the coupling steps required to get the two or more nucleobases of the blockmer incorporated into desired oligonucleotide. Finally, because they require fewer

- 34 -

condensation (coupling) steps, blockwise procedures should produce final reaction mixtures that contain fewer non-nucleobase containing contaminants (e.g., solvents) as well, a feature that further enhances the use of such procedures 5 with the methods and materials of the invention herein disclosed.

EXAMPLES

The following examples illustrate the invention and are not intended to limit the same. Those skilled in 10 the art will recognize, or be able to ascertain through routine experimentation, equivalents to the specific substances and procedures described. Such equivalents are considered to be within the scope of the present invention.

Example 1: Affinity Design

15 The matrix of the invention consists of several parts: a support (1), an optional linker (2) to the support, an optional spacer (3) and an affinity unit (4) designed to specifically hybridize to the target oligonucleotide. The probe of the matrix of the invention comprises parts (4) 20 and, optionally, (3). In Examples 1 to 3, all four parts are used. An aminohexyl group, a preferred linker (2) to the support (1), as it is easily attached to the 5' end of a oligonucleotide by a solid phase synthesizer, is used in Examples 1 to 3. In one embodiment of the invention, 25 oligo(dT)₅₋₃₀ acts as the optional spacer (3); in Examples 1 to 3, (dT)₁₅ is used. As described in more detail herein, an oligomer complementary to the target oligonucleotide in sequence is a preferred affinity unit.

As one example, a matrix to purify ISIS 2302,

30 5' -GCC-CAA-GCT-GGC-ATC-CGT-CA
SEQ ID NO:1.

is represented as

- 35 -

[SS] -NH₂-(CH₂)₆-T_n-5'-TGA-CGG-ATG-CCA-GCT-TGG-GC-3',

wherein the nucleotide sequence of the affinity unit is SEQ ID NO:2, "[SS]" indicates the solid support (1) and n = 5-30. The affinity unit of this matrix will hybridize with 5 ISIS 2302 to form a duplex of 20 base pairs (hybridization between bases is indicated by the "/" symbol):

SEQ ID NO:1

5' -GCCCAAGCTGGCATCCGTCA

|||||||||||||||||||

10 3' -CGGGTTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]

SEQ ID NO:2

Any (n-1), (n-2), etc. derivative of the desired oligonucleotide will have either one or more mismatches to the affinity unit or a shorter hybridizing sequence thereto.

15 The following representative deletion sequences serve as illustrations of this principle (deleted nucleotides relative to SEQ ID NO:1 are indicated by an asterisk, "*"):

5' -GCC-CAA-GCT-G*C-ATC-CGT-CA

SEQ ID NO:3,

20 5' -*CC-CAA-GCT-GGC-ATC-CGT-CA

SEQ ID NO:4,

5' -GCC-CAA-GCT-**C-ATC-CGT-CA

SEQ ID NO:5,

5' -GCC-CAA-*CT-G*C-ATC-CGT-CA

25 SEQ ID NO:6, and

5' -GCC-CAA-GCT-GG*-****-****-**

SEQ ID NO:7.

Potential compromised hybridization structures

- 36 -

with the affinity unit are represented as follows (matches, i.e., paired bases, are indicated by "|" and mismatched or unmatched bases are represented as "."):

For SEQ ID NO:3, probable hybridization structures

5 include

SEQ ID NO:3

5' -GCCCAAGCTGCATCCGTCA

|||||||.....|.....

3' -CGGGTTCGACCGTAGGGAGT-T_n- (CH₂)₆-NH₂- [SS]

10 SEQ ID NO:2,

a structure having only 11 "matches" (base pairs), or

SEQ ID NO:3
 5'-GCCCAAGCTG CATCCGTCA
 ||||||| |||||
 15 3'-CGGGTTCGAC GTAGGCAGT-T_n-(CH₂)₆-NH₂-[SS]
 \ /
 C
 SEQ ID NO:2,

20 a structure having 19 matches at the thermodynamic expense of forming a "bulge," i.e., excluding a single cytosine base from the structure. In any event, neither of these structures, nor any others that might be proposed, will comprise a 20 base pair hybridization structure as is found when the affinity unit binds oligonucleotide ISIS 2302 (SEQ 25 ID NO:1).

For SEQ ID NO:4, probable hybridization structures
include

SEQ ID NO:4
 5' - CCCAAGCTGGCATCCGTCA
 .|||||||||||||||||||
 3' - CGGGTTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 SEQ ID NO:2,

- 37 -

a structure having only 19 matches.

For SEQ ID NO:5, probable hybridization structures include

SEQ ID NO:5
5' -GCCCAAGCTCATCCGTCA
|||||.....
3' -CGGGTTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
SEQ ID NO:2,

a structure having only 9 matches, or

SEQ ID NO:5
5' -GCCCAAGCT CATCCGTCA
||||| | |||||
3' -CGGGTTCGA GTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
15 CC
SEQ ID NO:2,

a structure having 18 matches at the thermodynamic expense of forming a two base "bulge."

For SEQ ID NO:6, probable hybridization structures include

SEQ ID NO:6
5' - GCCCAACTGCATCCGTCA
...|.....| |||||
3' -CGGGTTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]

25 SEQ ID NO:2,

a structure having only 11 matches, or

SEQ ID NO:6
5' - GCCCAACTG CATCCGTCA
..|.|.| | |||||
30 3' - CGGGTTCGAC GTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
C
SEQ ID NO:2,

a structure having 15 matches at the thermodynamic expense of forming a one base "bulge," or

- 38 -

SEQ ID NO:6

5' -GCCCAA CTGCATCCGTCA
 ||||| | | |.....
 3' -CGGGTT GACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 5 | \ / |
 C
 SEQ ID NO:2,

a structure having 10 matches and at the thermodynamic expense of forming a one base "bulge," or

10 SEQ ID NO:6
 5' -GCCCAA CTG CATCCGTCA
 ||||| | | | | | | | |
 3' -CGGGTT GAC GTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 15 | \ / \ / |
 C C
 SEQ ID NO:2,

a structure having 18 matches at the thermodynamic expense of forming two one-base "bulges."

Finally, for SEQ ID NO:7, the probable
 20 hybridization structure is

SEQ ID NO:7
 5' -GCCCAAGCTGG
 ||||||| | | |.....
 3' -CGGGTTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 25 SEQ ID NO:2,

a structure having only 11 matches.

As will be appreciated by those skilled in the art, the hybridization structures with one or more one- or

- 39 -

two-base "bulges" are unstable because of the steric hindrance(s) and thermodynamic consequence(s) of excluding a base from the duplex. Accordingly, although not wishing to be bound by any theory in particular, the hybridization 5 structures that might preferentially form would be those having fewer matches but lacking "bulges."

Among undesired derivatives of the first oligonucleotide, purification of which is the object of the invention, the (n-1) deletion sequence with the terminal 10 base deleted is the hardest to separate from the full length oligonucleotide. In that case, perfect matches will be formed for the terminal (n-1) deletion sequence compared with n perfect matches for the full length oligonucleotides. All of the other sequences will have either fewer matches 15 and/or at least one mismatch, and, due to the formation of energetically disfavored duplexes with the affinity unit, thus can be separated from the full length oligonucleotide based on differential affinity.

Example 2: Preparation of Support:Activated Linker

20 Polystyrene, controlled pore glass (CPG) and polyethylene glycol (PEG), with PEG being reversibly precipitable, are some preferred supports. In this example, a solid support, a CPG bead with a terminal primary amine group (CPG, Inc., Lincoln Park, N.J.) was used as the 25 support (1) and linker (2). The CPG bead has a mean pore diameter of 569 D and a volume of 1.44 ml/g. The surface area is 55 m²/g, therefore, 10 mg of CPG beads has the same surface area as one 0.5 m x 0.5 m glass slide.

The primary amine of the linker was modified as 30 follows. 1,4-phenylene diisothiocyanate was dissolved in 100 ml pyrimine and 900 ml dimethyl formamide and reacted with 49.81 mg CPG at 37°C for 3 hours. The concentration of 1,4-phenylene diisothiocyanate was in large excess to minimize the dimer formation. The modified CPG was washed 35 once with acetone, twice with methanol, spun down to remove

- 40 -

the solvent, and allowed to dry. The linker of the resultant modified beads comprises a terminal isothiocyanate group.

Example 3: Immobilization of Probe

5 The probe, comprising the affinity unit (4) and optional spacer (3) is, in this example, an oligonucleotide that comprises a first oligonucleotide sequence, dT_n, where n is 15, that serves as the spacer, and a second oligonucleotide sequence, SEQ ID NO:2, that is the reverse 10 complement of the target oligonucleotide (ISIS 2302) and which serves as the affinity unit. The probe further comprises a primary aminohexyl group, -NH₂(CH₂)₆-, which is attached to the spacer at the 5' position of the terminal thymine residue.

15 To the tube containing the solid support:activated linker (Example 2) was added 0.5 ml of probe (145.9 nmol/ml) solution in 1x Tris buffer (pH 8.0). The mixture was incubated at 37°C for 4 hours. Under these mildly alkaline conditions, the primary amine group of the probe reacted 20 with the isothiocyanate group of the modified beads to form a thiocarbamyl adduct. The probe was thereby covalently attached to the surface of the solid support via the linker (Figure 2). Before and after immobilization, the concentration of the probe solution was measured by UV 25 absorbtion at 260 nm. The calculation indicated that 52.42 nmol of probe had been immobilized or adsorbed onto the CPG. The immobilized CPG was washed by distilled water and 3.6 nmol of the probe was eluted. Therefore, the amount of the probe immobilized onto the CPG was 48.82 nmol.

30 **Example 4: Hybridization, Extraction and Elution**

In this Example, hybridization (step (a)) was achieved by adding 1 ml of 10 fold diluted crude ISIS 2302 in 3x SSPE buffer solution to the immobilized CPG, followed by incubation at 37°C for 4 hours. By measuring the 35 absorbance of the solution before and after the

- 41 -

hybridization and after washing, it was determined that 46.9 nmol of ISIS 2302 had been hybridized to the probe on the support. A preferred hybridization structure is represented as follows:

5 SEQ ID NO:1
5' -GCCCAAGCTGGCATCCGTCA-3'
 |||||||||||||||||||
3' -CGGGTTCGACCGTAGGCAGT-T₁₅-(CH₂)₆-NH₂-[CPG]
SEQ ID NO:2

10 The ratio of target oligonucleotide to the probe was 0.9607, that is to say, the efficiency of hybridization is 96%, or close to 1:1.

In some instances, it may be desirable to rinse the bound oligonucleotides before the recovery step (c) in 15 order to enhance the removal of undesired derivative oligonucleotides or any other impurities. For example, the hybridized CPG may be washed once or twice with the extraction buffer. In this example, the removal (step (b)) of undesired oligonucleotides was accomplished as follows.

20 The bound oligonucleotides were rinsed with 0.5 ml of 2x SSPE buffer. The rinse was performed 2x at 37°C.

After hybridization (step (a)) and removal of undesired and unbound contaminants (step (b)), hybridized ISIS 2302 was dissociated and recovered (step (c)) by 25 incubation with distilled water at 90°C for 30 minutes. As is known by those skilled in the art, there are several means of dissociating bound synthetic oligonucleotide from the affinity unit of the invention. For example, a pH gradient could be used, starting at about pH 5 and finishing 30 at about pH 10. Under these conditions, most target oligonucleotides and affinity units will be stable, and the pH of the resultant purified oligonucleotide can be easily adjusted before further use by, for example, dialysis. It will be further appreciated by those skilled in the art that 35 the means of dissociating and recovering bound target

- 42 -

oligonucleotide will vary according to the chemical nature of the matrix and affinity unit being used. For example, the affinity unit may be or comprise a peptide nucleic acid (PNA). Because of the neutral charge of PNA, ammonium hydroxide can be added, at a concentration of from about 0.5% to about 10% (wt/vol), to a matrix to which target oligonucleotide is bound in order to effect dissociation of the target oligonucleotide from the matrix. Ammonium hydroxide can then be removed from the purified target 5 oligonucleotide according to methods known in the art.

10

Example 5: Purity Analysis

The purity of the purified full length oligonucleotide was measured by capillary gel electrophoresis (CGE). Both crude and extracted ISIS 2302 15 solutions were analyzed by CGE. The resultant electropherograms (Figure 3, crude ISIS 2302; Figure 4, extracted ISIS 2303) show that the purity of the extracted oligonucleotide solution is much higher than the original one. The earlier eluting (n-1), (n-2), etc. undesired 20 derivative oligonucleotides are to the left of the large, narrow peak of desired target oligonucleotide. Integration of the areas under the peaks indicates that the crude ISIS 2302 preparation contains 13.5% undesired derivative oligonucleotides. In contrast, ISIS 2302 purified by the 25 method of the invention contains only 2.8% undesired derivative oligonucleotides. Thus, the purity of the desired target oligonucleotide has been increased from 86.5% to 97.2%.

To compare the results and evaluate the method of 30 the invention, crude ISIS 2303, HPLC-purified ISIS 2303 and ISIS 2303 purified according to the method of the invention are electrophoretically separated under the same conditions.

Example 6: Modified Affinity Units

In this embodiment of the invention, one or more 35 nucleotides of the affinity unit comprises at least one

chemical modification which (i) lowers the affinity of the probe for one or more undesired oligonucleotides but does not adversely impact the probe's affinity for the desired oligonucleotide, (ii) raises the affinity of the probe for 5 the target oligonucleotide but does not enhance the probe's affinity for the undesired oligonucleotides, or (iii) achieves both of goals (i) and (ii).

Specific examples of some modified oligonucleotides that can be incorporated into the probe 10 include those containing phosphorothioates, phosphotriesters, methyl phosphonates, short chain alkyl or cycloalkyl intersugar linkages or short chain heteroatomic or heterocyclic intersugar linkages. Specifically, oligonucleotides with phosphorothioates and those with CH₂- 15 NH-O-CH₂, CH₂-N(CH₃)-O-CH₂ [known as a methylene(methylimino) or MMI backbone], CH₂-O-N(CH₃)-CH₂, CH₂-N(CH₃)-N(CH₃)-CH₂ and O-N(CH₃)-CH₂-CH₂ backbones, wherein the native phosphodiester backbone is represented as O-P-O-CH₂), oligonucleotides having morpholino backbone structures (Summerton and Weller, 20 U.S. Patent 5,034,506) or a peptide nucleic acid (PNA) backbone (in which the phosphodiester backbone of the oligonucleotide is replaced with a polyamide backbone, the nucleobases being bound directly or indirectly to the aza nitrogen atoms of the polyamide backbone (Nielsen et al., 25 Science, 1991, 254, 1497), modified oligonucleotides containing one or more substituted sugar moieties [i.e., sugar moieties comprising one of the following at the 2' position: OH, SH, SCH₃, F, OCN, OCH₂OCH₃, OCH₂O(CH₂)_nCH₃, O(CH₂)_nNH₂ or O(CH₂)_nCH, where n is from 1 to about 10; C₁ to 30 C₁₀ lower alkyl, alkoxyalkoxy, substituted lower alkyl, alkaryl or aralkyl; Cl; Br; CN; CF₃; OCF₃; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; SOCH₃; SO₂CH₃; ONO₂; NO₂; N₃; NH₂; heterocycloalkyl; heterocycloalkaryl; aminoalkylamino; polyalkylamino; substituted silyl; an RNA cleaving group; a 35 reporter group; an intercalator; a group for improving the pharmacokinetic properties of an oligonucleotide; or a group for improving the pharmacodynamic properties of an

- 44 -

oligonucleotide and other substituents having similar properties], including 2'-O-methoxyethoxy [2'-O-CH₂CH₂OCH₃, also known as 2'-O-(2-methoxyethyl)] (Martin et al., *Helv. Chim. Acta*, 1995, 78, 486), 2'-methoxy (2'-O-CH₃), 2'-propoxy (2'-OCH₂CH₂CH₃) and 2'-fluoro (2'-F), or similar modifications made at other positions on the oligonucleotide (particularly the 3' position of the sugar on the 3' terminal nucleotide and the 5' position of 5' terminal nucleotide), 5 oligonucleotides having sugar mimetics such as cyclobutyls in place of the pentofuranosyl group or base modifications 10 or substitutions (e.g., with a "universal" base such as inosine) can be used in this embodiment.

The target oligonucleotide may also comprise one or more the above modifications. A further modification of 15 the target oligonucleotide involves chemically linking to the target oligonucleotide one or more lipophilic moieties which enhance the cellular uptake of the oligonucleotide. Such lipophilic moieties include but are not limited to a cholesteryl moiety (Letsinger et al., *Proc. Natl. Acad. Sci. USA*, 1989, 86, 6553), cholic acid (Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1994, 4, 1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., *Ann. N.Y. Acad. Sci.*, 1992, 660, 306; Manoharan et al., *Bioorg. Med. Chem. Lett.*, 1993, 3, 2765), a thiocholesterol (Oberhauser et al., *Nucl. Acids Res.*, 1992, 20, 533), an aliphatic chain, e.g., dodecandiol 20 or undecyl residues (Saison-Behmoaras et al., *EMBO J.*, 1991, 10, 111; Kabanov et al., *FEBS Lett.*, 1990, 259, 327; Svinarchuk et al., *Biochimie*, 1993, 75, 49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di- 25 30 O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., *Tetrahedron Lett.*, 1995, 36, 3651; Shea et al., *Nucl. Acids Res.*, 1990, 18, 3777), a polyamine or a polyethylene glycol chain (Manoharan et al., *Nucleosides & Nucleotides*, 1995, 14, 969), or adamantan acetic acid (Manoharan et al., 35 *Tetrahedron Lett.*, 1995, 36, 3651), a palmityl moiety (Mishra et al., *Biochim. Biophys. Acta*, 1995, 1264, 229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol.

- 45 -

moiety (Crooke et al., *J. Pharmacol. Exp. Ther.*, 1996, 277, 923). Oligonucleotides comprising lipophilic moieties, and methods for preparing such oligonucleotides, are disclosed in U.S. Patents No. 5,138,045, No. 5,218,105 and No.

5 5,459,255.

The target oligonucleotide, an oligonucleotide present in the probe, or both, can also be oligonucleotides which are chimeric oligonucleotides including "gapmers" and "hemimers." Chimeric oligonucleotides are oligonucleotides 10 which contain two or more chemically distinct regions, each made up of at least one nucleotide. These oligonucleotides typically contain at least one terminal region wherein the oligonucleotide is modified so as to confer upon the oligonucleotide increased resistance to nuclease 15 degradation, increased cellular uptake, and/or increased binding affinity for the intracellular target nucleic acid. In a "hemimer," a single terminal (either 5' or 3') region is so modified in the oligonucleotide structure. When both termini of the oligonucleotide are modified, the 20 oligonucleotide is called a "gapmer" and the modified 5'- and 3'-terminal regions are referred to as "wings"; an additional, typically central, region (typically referred to as the "gap" or "core") of the oligonucleotide may serve as a substrate for cellular enzymes capable of cleaving RNA:DNA 25 or RNA:RNA hybrids. In a gapmer, the 5' and 3' wings can be modified in the same or different manner depending on what properties it is desired to achieve.

A preferred modification in many chimeric oligonucleotides designed for antisense purposes is the 30 inclusion of one or more residues modified at the 2' position. An example is the 2'-O-methyl modification, which, when incorporated into synthetic oligonucleotides, results in increased duplex stability (nearly 0.8 kcal/mol per modification) between such oligonucleotides and RNA. 35 targets. At the same time that it enhances the duplex stability between the oligonucleotide and an RNA target, however, the 2'-O-methyl modification slightly destabilizes

- 46 -

the duplex formed between the oligonucleotide and a DNA target (Freier, Chapter 5 In: *Antisense Research and Applications*, Crooke et al., Eds., 1993, CRC Press, Boca Raton, LA, pages 67-82).

5 Modifications that enhance the affinity for, and/or duplex stability with, RNA molecules are preferred for applications wherein such oligonucleotides are intended for antisense purposes, wherein the oligonucleotide is designed to selectively bind to, and modulate the expression 10 of, a particular RNA species. In the methods of the invention, affinity units comprising nucleobase sequences complementary to such modified portions of target oligonucleotides are designed to reflect the chemical modification(s) present in the target oligonucleotide. In 15 the case of 2'-O-methyl-modified oligonucleotides, for example, an affinity unit comprising RNA or RNA-like nucleotide units would, due to the differences in duplex stability between RNA and DNA targets, be preferable to one composed strictly of deoxyribonucleotides.

20 By "RNA-like" it is meant that such an oligonucleotide, or such portion thereof, has an ability to form a stable duplex with an RNA target that exceeds its ability to form a stable duplex with a DNA target under comparable conditions. The acronym "RLO" refers to "RNA- 25 like oligonucleotide" herein. While not wishing to be bound by any particular theory, it is presently believed that RNA:RNA, RNA:RLO and RLO:RLO duplexes differ from DNA:DNA and DNA:RNA duplexes in several significant conformational respects (Lesnik et al., *Biochemistry*, 1993, 32, 7832; 30 Lesnik et al., *Biochemistry*, 1995, 34, 10807), and that these conformational differences contribute to the relative stabilities of structurally distinct duplex partners. In any event, target antisense oligonucleotides (ASO's) comprising an RNA or RNA-like hybridizing portion are 35 preferentially purified using a matrix having an affinity unit comprising a hybridizing portion with one or more the following modifications, which enhance the stability of

- 47 -

duplexes formed with such modified affinity units and RNA or RLO molecules, and/or enhance the affinity of such modified affinity units for RNA or RLO molecules, and are thus preferred. Conversely, target ASO's comprising one or more 5 of the following modifications in their hybridizing portion are preferably purified using affinity units having an RNA or RNA-like structure in their corresponding hybridizing portions. For this embodiment, such preferred modifications include but are not limited to sugar modifications, backbone 10 modifications, nucleobase modifications or combinations thereof.

A preferred group of sugar modifications are modified at the 2' position. These include 2'-fluoro substitutions; 2'-O-alkyl substitutions, particularly those 15 with relatively small (i.e., C₁-C₁₀) substituent groups, e.g., 2'-O-allyl (2'-O-CH₂-CH=CH₂), 2'-O-butyl [2'-O-(CH₂)₃CH₃] and 2'-O-methyl (2'-O-CH₃) substitutions; 2'-O-methoxy-ethyl, (2'-O-CH₂CH₂-O-CH₃) substitutions; 2'-O-alkoxy-alkoxy [2'-O-(CH₂CH₂O)_n(CH₂)_mCH₃] substitutions, where m is from 0 to 6 and n 20 is from 2 to 6; 2'-O-aminoalkyl [2'-O-(CH₂)_nNH₂]; 2'-O-CH₂-CHR-X where X = OH, F, CF₃ or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃; and an intercalating substituent, such as that present on 2'-O-anthraquinolylmethyl uridine. Other sugar 25 modifications include, for example, 4'-6' methano carbocyclic derivatives.

Backbone modifications (modified linkages) include the polyamide backbone, PNA (peptide nucleic acid); -CH₂-CO-NCH₂-CH₂-; the methylene(methylimino) or MMI backbone (-CH₂-NCH₃-O-CH₂-); the dimethylhydrazino or MDH backbone (-CH₂-NCH₃-NCH₃-CH₂-); amide 3 (-CH₂-CO-NH-CH₂-) or amide 4 (-CH₂-NH-CO-CH₂-)-based backbones; phosphoryl linked morpholino 30 backbone; phosphonate (-CH₂-PO₂X-O-, where X=O or S) linkages such as, e.g., those disclosed in U.S. Patent No. 5,610,289; formacetal/ketal type linkages, such as, for example, those 35 disclosed in U.S. Patent No. 5,264,562; and backbones incorporating HNA (1,5-anhydrohexitol) (Herdewijn, *Liebigs Ann.*, 1996, 1337; published PCT patent application WO

96/05213).

Nucleobase modifications include 5-methyl-cytosine and uridine and thymine derivatives, including those having substitutions of the 5-methyl group, e.g., by a propynyl (-5 C≡C-CH₃) methylthiazole or amino-ethyl-3-acrylimido substituent and 2-thio uridine or thymidine. A combination of 2'-fluoro-5-propynyl deoxyuridine is especially preferred. Preferred purine modifications include 7-modified-7-deaza purines and 2-amino-adenosine.

10 Certain combinations of the above modifications are also preferred in this embodiment of the invention. These include, but are not limited to, amide 3 or MMI modified linkages having a 2'-O-methyl group on the "lower" sugar (i.e., the sugar 3' of the modified linkage) and amide 15 3 and MMI modified linkages having 2'-O-methyl groups on both the "upper" sugar (i.e., the sugar 5' of the modified linkage) and the lower sugar; MMI modified linkages having 2'-fluoro substitutions on the lower, upper or both sugars; oligonucleotides having alternating MMI and phosphodiester 20 linkages, which have the properties of high nuclease resistance as well as enhanced affinity for and duplex stability with RNA and RNA-like targets; MMI modified linkages having 2' substitutions on both the upper and lower sugar that are different substitutions (for example, 2'-O- 25 methyl on the upper sugar and 2'-fluoro on the lower sugar); MMI modified linkages having one or more 2'-methoxy ethyl substitutions; and 2'-fluoro, N3'->P5' phosphoramidite oligonucleotides (Schultz et al., *Nucleic Acids Res.*, 1996, 24, 2966).

30 For this embodiment of the invention, particularly preferred nucleobases include, but are not limited to, 2'-fluoro-propynyl uridine, substituted for uridine or thymine, and 2'-O-methyl, 2-amino-adenosine in substitution for adenosine. Among oligonucleotide-based affinity units, 35 particularly preferred are 2'-O-methyl MMI backbones, 2'-O-methyl amide 3 backbones, and 2'-fluoro, N3'->P5' phosphoramidite oligonucleotides. While not wishing to be

bound by any particular theory, it has been suggested that oligonucleotides comprising certain of these modifications (e.g., amide 3 and amide 4) have the tendency to organize themselves, prior to hybridization, into conformations more favorable for duplex formation. In other instances, and again not wishing to be bound by any particular theory, it has been proposed that certain of these modifications favor the C3' endo pucker conformation which, it is believed, RNA:RNA and RNA:RLO duplexes adopt (Lesnik et al., 5 *Biochemistry*, 1993, 32, 7832; Kawasaki et al., *J. Med. Chem.*, 1993, 36, 831; Griffey et al., *In: carbohydrate Modifications in Antisense Research*, Sagvhi et al., eds., ACS Symp. Ser. 580, Amer. Chem. Socy., Washington, D.C., 1994, 10 pp. 212-224). Yet another contribution, particularly in the case of nucleobase modifications, may come from other 15 stabilizing effects, such as improved base stacking (Froehler et al., *Tetrahedron Letts.*, 1992, 37, 5307), although applicants do not wish to be bound by any 20 particular theory or theories regarding such effects. It will also be appreciated by those skilled in the art that increased affinity between the hybridizing portions of the target oligonucleotide is desirable only up to the point 25 where discrimination between properly matched and mismatched species begins to deteriorate. Those of ordinary skill in the art will be able to prepare particular applications of the invention wherein the affinity and duplex stability of the matrix of the invention specific are maximized while an acceptable degree of discrimination is retained.

The choice of modification(s) to be incorporated 30 into the affinity unit is thus influenced by the chemical nature of the target oligonucleotide. Furthermore, modifications can be strategically placed within the affinity unit in order to maximize the separation of undesired oligonucleotides that are particularly refractory 35 to separation from the desired full length oligonucleotide. For example, the hybridization structure of Example 4 is modified in the following manner:

SEQ ID NO:1

5' - ~~*****~~ G~~CCCAAGCTGGCATCCGTCA~~ -~~*****~~ -3'

||||| | | | | | | | | | | | | | |

5 3' - CGGGTTCGACCGTAGGCAGT -T₁₅ - (CH₂)₆-NH₂ - [CPG]

SEQ ID NO:2,

where the underlined nucleotides in the affinity unit (SEQ ID NO:2) are ribonucleotides rather than deoxyribonucleotides, and the target oligonucleotide (SEQ ID

10 NO:1) comprises terminal 2'-O-methoxyethoxy modifications, as indicated by the asterisks. It is known in the art that, in DNA duplexes, the presence of 2'-O-methoxyethoxy modifications has a slight negative effect on duplex stability; in contrast, in DNA:RNA duplexes, the 2'-O-methoxyethoxy modification increases duplex stability by about 0.8 kcal/mol per modification (Freier, chapter 5 in: *Antisense Research and Applications*, Crooke et al., eds., CRC Press, Boca Raton, 1993, page 69). Thus, as a consequence of the choice of modifications to the target

15 oligonucleotide and the affinity unit, the above hybridization structure has a significantly greater stability when bound to the desired target chimeric oligonucleotide than when bound to undesired (n-1) derivatives lacking a single terminal nucleotide, i.e.,

20

25 SEQ ID NO:1

5' - ~~***~~ C~~CCCAAGCTGGCATCCGTCA~~ -~~***~~ -3'

| | | | | | | | | | | | | |

30 3' - CGGGTTCGACCGTAGGCAGT -T₁₅ - (CH₂)₆-NH₂ - [CPG]

SEQ ID NO:2

and

SEQ ID NO:1

5' - ~~***~~ G~~CCCAAGCTGGCATCCGTCA~~ -~~***~~ -3'

| | | | | | | | | | | | | |

35 3' - CGGGTTCGACCGTAGGCAGT -T₁₅ - (CH₂)₆-NH₂ - [CPG]

SEQ ID NO:2.

Although these differences in affinity are also present when the affinity unit is not so modified, the degree of differential affinity is enhanced due to the modifications. In the above exemplars, the desired hybridization structure 5 is expected to have an increased stability of about 0.8 kcal/mol when compared with either of the duplexes which incorporate an undesired terminal (n-1) oligonucleotide.

Example 7: Essentially Full-Length Affinity Oligonucleotides

Although the nucleobase sequence of the affinity 10 unit may be "full-length", i.e., of the same length, n, as the target oligonucleotide, affinity units having "essentially full-length" nucleobase sequences may also be used. In this embodiment of the invention, the affinity unit has a nucleobase sequence that is of a length, p, that 15 is less than the length, n, of the target oligonucleotide. However, the nucleobase sequence of the affinity unit is nonetheless the reverse complement of the target oligonucleotide over length p. Moreover, p is a number ranging from n-4 to n, wherein, in a duplex between the 20 target oligonucleotide and the nucleobase sequence of the affinity unit, neither the 5' overhang nor the 3' overhang of said target oligonucleotide is greater than two nucleotides.

As exemplars, affinity units having essentially 25 full-length nucleotide sequences for ISIS 2302 are given as SEQ ID NOS:8 to 15. These affinity oligonucleotides will form the following duplexes with the target oligonucleotide, ISIS 2302 (n = 20 nucleotides). For SEQ ID NOS: 8 and 9 (p = 19 nucleotides),

30 SEQ ID NO:1
 5' - GCCCAAGCTGGCATCCGTCA
 .|||||||||||||||||||
 3' - GGGTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 SEQ ID NO:8;

35 and

SEQ ID NO:1
5' -GCCCAAGCTGGCATCCGTCA
||||||||||||||||||.
3' -CGGGTTCGACCGTAGGCAG -T_n-(CH₂)₆-NH₂- [SS]
5 SEQ ID NO:9.

For SEQ ID NOS: 10 to 12 (p = 18 nucleotides),

and

SEQ ID NO:1
 5' - GCCCAAGCTGGCATCCGTCA
 20 ..|||||||||||||||||||
 3' - GGTCGACCGTAGGCAGT-T_n-(CH₂)₆-NH₂- [SS]
 SEQ ID NO:12.

For SEQ ID NOS: 13 and 14 (p = 17 nucleotides),

SEQ ID NO:1
25 5' -GCCCAAGCTGGCATCCGTCA
.....
3' - GGGTCGACCGTAGGCA - T_n - (CH₂)₆-NH₂ - [SS]
SEQ ID NO:13;

- 53 -

and

SEQ ID NO:1
5' -GCCCAAGCTGGCATCCGTCA
..|||||||||||||||..
5 3' - GGTCGACCGTAGGCAG -T_n-(CH₂)₆-NH₂- [SS]
SEQ ID NO:14.

Finally, for SEQ ID NO:15 (p = 16 nucleotides),

SEQ ID NO:1
5' -GCCCAAGCTGGCATCCGTCA
..|||||||||||||||..
10 3' - GGTCGACCGTAGGCA -T_n-(CH₂)₆-NH₂- [SS]
SEQ ID NO:15.

It will be appreciated by those skilled in the art that certain parameters (e.g., temperature of elution and extraction, etc.) will have to be adjusted to compensate for the loss of nucleobase partners in the affinity unit. Methods for estimating and determining these parameters are known in the art (see, for example, Lehninger, *Biochemistry*, 2nd Ed., 1970, Worth Publishers Inc., New York, NY, page 15 20 875; Jarrett, *J. Chromatogr.*, 1993, 618, 315; Freier, Chapter 5 In: *Antisense Research and Applications*, Crooke et al., Eds., 1993, CRC Press, Boca Raton, LA). Moreover, if desired, the presence of oligonucleotides in the flow-through during the extraction step (b) or elution step (c) 25 can be monitored by a variety of methods known in the art. See, for example, Jarrett, *J. Chromatogr.*, 1993, 618, 315.

Example 8: Repeated Rounds of Purification Using Different Affinity Units

In some instances, it may be desirable to carry 30 out multiple rounds of purification of a desired target

- 54 -

oligonucleotide by different matrices developed according to the present invention. As one example, for a longer target oligonucleotide, it may be difficult or expensive to develop one affinity unit, corresponding to an extended hybridizing portion of such a target oligonucleotide, of sufficient

length and/or purity. Purification of a long oligonucleotide using an affinity unit that is less than full-length may result in the inclusion of mismatched derivatives that comprise mismatches (* in the diagram 10 below) outside of the hybridizing portion of the target oligonucleotide:

First Affinity

Target oligonucleotide

(bound)

15

Mismatched oligonucleotides not bound/retained are diagrammed as follows:

First Affinity

Unit _____

A horizontal scale bar consisting of a line with 12 vertical tick marks. The 7th tick mark from the left is marked with a small asterisk (*).

||||*|||||, etc.

Mismatched oligonucleotides potentially bound/retained are diagrammed as follows:

25 First Affinity

Unit _____

— * —

- 55 -

|||||||||||*|, etc.

By applying the partially purified target oligonucleotide resulting from contacting and processing through a matrix comprising a first affinity unit to a 5 matrix comprising a second affinity unit, wherein the first and second affinity units are complementary to different hybridizing portions of the target oligonucleotide, the mismatched oligonucleotides potentially bound and/or retained from the matrix comprising the first affinity unit 10 are removed:

| | | |
|----|---|--------------------------------|
| | | Second Affinity Unit |
| | | Target oligonucleotide (bound) |
| | * | Mismatched oligonucleotides |
| 15 | * | from First Affinity Unit (not |
| | * | bound/retained by Second |
| | * | Affinity Unit) |

Although, in the above example, the affinity units are complementary to hybridizing portions of the target 20 oligonucleotide located at 5' and 3' termini, no particular placement of the hybridizing portions is intended, other than that such hybridizing portions of the target oligonucleotide should typically be distinct from one another so that different undesirable contaminating 25 derivatives of the target oligonucleotide are removed by each matrix. Thus, the first and second hybridizing portions can each be located at a 5' terminus, a 3' terminus, or within a central portion of the target oligonucleotide.

30 The temporal ordering of the two purification steps is not typically important; in the above scheme, for example, the "Second Affinity Unit" might just as well be used before the "First Affinity Unit" in most instances. Temporal ordering of the two purification steps may be a

- 56 -

consideration, however, when the matrices comprising the two affinity units are of different chemical composition and/or depending on the means used to dissociate and recover target oligonucleotide from the first affinity unit to which it is 5 bound. For example, consider an instance wherein the eluent from affinity unit "A" is prepared in such a way so as to

include one or more components (e.g., salts, solvents, ions, etc.) that would chemically disrupt the matrix into which affinity unit "B" is incorporated. In the latter instance, 10 if the eluent from affinity unit "B" does not have such an effect on the matrix comprising affinity unit "A," it would be preferable to apply the mixture comprising the target oligonucleotide to matrix/affinity unit "B" before applying it to matrix/affinity unit "A."

15 It should also be appreciated by those skilled in the art that the number of affinity units and stepwise purifications need not be limited to two and that, in some instances, several such stepwise purifications, and appropriate affinity units and matrices, may be needed.

20 Moreover, the two affinity units can be of the same or different chemical composition, depending on the chemical nature of the target oligonucleotide. For example, for a "hemimer" having a 3' portion comprising 2'-O-methoxy ethyl modified residues and a 5' portion comprising standard

25 deoxyribonucleotide residues, the affinity unit for the RNA-like 3' hybridizing portion would preferably incorporate one or more of the modifications detailed in Example 6, whereas no such preference would exist for an affinity unit designed to hybridize to the DNA-like 5' portion of such an

30 oligonucleotide. In fact, as will be appreciated by those skilled in the art, some of the modifications presented in Example 6 (e.g., 2'-O-methyl) actually lower the ability of the affinity unit to bind DNA and would thus be disfavored for affinity units designed to interact with a DNA-like 35 hybridizing portion of a target oligonucleotide.

- 57 -

It will be further appreciated by those skilled in the art that the examples and embodiments described herein are for illustrative purposes only. Various modifications in light the disclosure will be suggested to persons skilled 5 in the art and are intended to be included within the purview of the application and the scope of the claims.

- 58 -

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Isis Pharmaceuticals, Inc. et al.
(ii) TITLE OF INVENTION: Large-Scale Purification of
5 Full Length Oligonucleotides by Solid-Liquid
Affinity Extraction

(iii) NUMBER OF SEQUENCES: 243

(iv) CORRESPONDENCE ADDRESS:

10 (A) ADDRESSEE: Woodcock Washburn Kurtz Mackiewicz
& Norris
(B) STREET: One Liberty Place - 46th Floor
(C) CITY: Philadelphia
(D) STATE: PA
(E) COUNTRY: USA
15 (F) ZIP: 19103

(v) COMPUTER READABLE FORM:

20 (A) MEDIUM TYPE: DISKETTE, 3.5 INCH, 1.44 MB
STORAGE
(B) COMPUTER: IBM PS/2
(C) OPERATING SYSTEM: PC-DOS
(D) SOFTWARE: WORDPERFECT 6.1

(vi) CURRENT APPLICATION DATA:

25 (A) APPLICATION NUMBER: n/a
(B) FILING DATE: Herewith

(vii) PRIOR APPLICATION DATA:

30 (A) APPLICATION NUMBER 08/769,951
(B) FILING DATE December 19, 1996

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: John W. Caldwell
30 (B) REGISTRATION NUMBER: 28,937
(C) REFERENCE/DOCKET NUMBER: ISIS-2741

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: (215) 568-3100
(B) TELEFAX: (215) 568-3439

- 59 -

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

- (D) OTHER INFORMATION: ISIS 2302

10 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER:

(I) FILING DATE:

(J) PUBLICATION DATE:

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

15 GCCCAAGCTG GCATCCGTCA

20

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 1

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

TGACGGATGC CAGCTTGGGC

20

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 19 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 60 -

(D) OTHER INFORMATION: Deletion derivative of SEQ
ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
GCCCAAGCTG CATCCGTCA

19

5 (2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Deletion derivative of SEQ
ID NO: 1

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CCCAAGCTGG CATCCGTCA

19

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
20 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Deletion derivative of SEQ
ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GCCCAAGCTC ATCCGTCA

18

(2) INFORMATION FOR SEQ ID NO: 6:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

- 61 -

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Deletion derivative of SEQ ID NO: 1

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:
GCCCAACTGC ATCCGTCA 18

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 base pairs

10 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

15 (D) OTHER INFORMATION: Deletion derivative of SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:
GCCCAAGCTG G 11

(2) INFORMATION FOR SEQ ID NO: 8:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

25 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Essentially full-length reverse complement of SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
30 TGACGGATGC CAGCTTGCG 19

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: Nucleic Acid

- 62 -

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

5 (D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GACGGATGCC AGCTTGGC

19

(2) INFORMATION FOR SEQ ID NO: 10:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

15 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

20 GACGGATGCC AGCTTGGG

18

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

25 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

ACGGATGCCA GCTTGGC

18

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- 63 -

(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

5 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:
10 TGACGGATGC CAGCTTGG 18

(2) INFORMATION FOR SEQ ID NO: 13:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
15 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1
20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
ACGGATGCCA GCTTGGG 17

(2) INFORMATION FOR SEQ ID NO: 14:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
30 (ix) FEATURE:
(D) OTHER INFORMATION: Essentially full-length
reverse complement of SEQ ID NO: 1
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
GACGGATGCC AGCTTGG 17

- 64 -

(2) INFORMATION FOR SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs

(B) TYPE: Nucleic Acid

5 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Essentially full-length
10 reverse complement of SEQ ID NO: 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

ACGGATGCCA GCTTGG

16

(2) INFORMATION FOR SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to c-myb mRNA;
a.k.a. "MYB-AS"

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Calabretta, Bruno, et al.

25 (B) TITLE: Inhibition of Protooncogene Expression
in Leukemic Cells: An Antisense Approach

(C) JOURNAL: Antisense Research and Applications,
Crooke, S.T., et al., eds., CRC Press, Boca Raton

(D) VOLUME: Chapter 31

30 (F) PAGES: 535-545

(G) DATE: 1993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

GTGCCGGGGT CTTCGGGC

18

(2) INFORMATION FOR SEQ ID NO: 17:

35 (i) SEQUENCE CHARACTERISTICS:

- 65 -

(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
5 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 16
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
10 GCCCGAAGAC CCCGGCAC

18

(2) INFORMATION FOR SEQ ID NO: 18:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
15 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to mammalian DNA
20 methyl transferase
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/15378 (SEQ ID NO: 1)
(I) FILING DATE: 30-NOV-1994
(J) PUBLICATION DATE: 08-JUN-1995
25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:
CATCTGCCAT TCCCACTCTA

20

(2) INFORMATION FOR SEQ ID NO: 19:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
30 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse

- 66 -

complement of SEQ ID NO: 18

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:
TAGAGTGGGA ATGGCAGATG

20

(2) INFORMATION FOR SEQ ID NO: 20:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to mammalian DNA
methyl transferase

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 95/15378 (SEQ ID NO: 2)

(I) FILING DATE: 30-NOV-1994

(J) PUBLICATION DATE: 08-JUN-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

TTGGCATCTG CCATTCCAC TCTA

24

20 (2) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 20

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

TAGAGTGGGA ATGGCAGATG CCAA

24

(2) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

- 67 -

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- 5 (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)
- (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 1)
 - 10 (I) FILING DATE: 26-JUL-1994
 - (J) PUBLICATION DATE: 09-FEB-1995
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:
CATGGTTTCG GAGGGCGTC
- 19

- (2) INFORMATION FOR SEQ ID NO: 23:
- 15 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 19 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- 20 (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 22
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:
GACGCCCTCC GAAACCATG
- 25 19

- (2) INFORMATION FOR SEQ ID NO: 24:
- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 21 base pairs
 - (B) TYPE: Nucleic Acid
 - 30 (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF); a.k.a. "Vm"
- 35

- 68 -

5 (x) PUBLICATION INFORMATION:

(A) AUTHORS: Robinson, G.S., et al.
(B) TITLE: Oligodeoxynucleotides inhibit retinal neovascularization in a murine model of proliferative retinopathy
(C) JOURNAL: The Proceedings of the National Academy of Sciences (U.S.A.)
(D) VOLUME: 93
(F) PAGES: 4851-4856
(G) DATE: MAY-1996

10 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 2)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

CAGCCTGGCT CACCGCCTTG G

21

(2) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 24

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

CCAAGGCGGT GAGCCAGGCT G

21

(2) INFORMATION FOR SEQ ID NO: 26:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: Yes

- 69 -

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Vascular
Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

5 (A) AUTHORS: Robinson, G.S., et al.

(B) TITLE: Oligodeoxynucleotides inhibit retinal
neovascularization in a murine model of
proliferative retinopathy (SEQ ID NO: M3)

10 (C) JOURNAL: The Proceedings of the National
Academy of Sciences (U.S.A.)

(D) VOLUME: 93

(F) PAGES: 4851-4856

(G) DATE: MAY-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

15 TCGCGCTCCC TCTCTCCGGC

20

(2) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

20 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 26

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GCCGGAGAGA GGGAGCGCGA

20

(2) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 70 -

(D) OTHER INFORMATION: Antisense to Vascular
Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 4)

5 (I) FILING DATE: 26-JUL-1994

(J) PUBLICATION DATE: 09-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

CACCCAAGAG AGCAGAAAGT

20

(2) INFORMATION FOR SEQ ID NO: 29:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

15 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 28

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

20 ACTTTCTGCT CTCTTGGGTG

20

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

(B) TYPE: Nucleic Acid

25 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Vascular
Endothelial Growth factor (VEGF)

30 (x) PUBLICATION INFORMATION:

(A) AUTHORS: Nomura, M., et al.

(B) TITLE: Possible Participation of Autocrine and
Paracrine Vascular Endothelial Growth factors in
35 Hypoxia-induced Proliferation of Endothelial Cells

- 71 -

and Pericytes

(C) JOURNAL: The Journal of Biological Chemistry
(D) VOLUME: 270
(E) ISSUE 47
5 (F) PAGES: 28316-28324
(G) DATE: 24-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:
CCCAAGACAG CAGAAAGTTC AT

22

(2) INFORMATION FOR SEQ ID NO: 31:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
15 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 30
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:
20 ATGAACTTTC TGCTGTCTTG GG

22

(2) INFORMATION FOR SEQ ID NO: 32:

25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Vascular
30 Endothelial Growth factor (VEGF)
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 5)
(I) FILING DATE: 26-JUL-1994
(J) PUBLICATION DATE: 09-FEB-1995
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

- 72 -

TCGTGGGTGC AGCCTGGGAC

20

(2) INFORMATION FOR SEQ ID NO: 33:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 33:

GTCCCAGGCT GCACCCACGA

20

(2) INFORMATION FOR SEQ ID NO: 34:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

20 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

25 (H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 11)

(I) FILING DATE: 26-JUL-1994

(J) PUBLICATION DATE: 09-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 34:

CTGCCCGGCT CACCGCCTCG G

21

30 (2) INFORMATION FOR SEQ ID NO: 35:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single

- 73 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 34

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 35:
CCGAGGCGGT GAGCCGGCA G 21

(2) INFORMATION FOR SEQ ID NO: 36:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 19 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

15 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Vascular Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 12)

20 (I) FILING DATE: 26-JUL-1994

(J) PUBLICATION DATE: 09-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 36:
CATGGTTTCG GAGGCCCGA 19

(2) INFORMATION FOR SEQ ID NO: 37:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

30 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 36

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 37:
35 TCGGGCCTCC GAAACCATG 19

- 74 -

(2) INFORMATION FOR SEQ ID NO: 38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to Vascular
10 Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 13)
- (I) FILING DATE: 26-JUL-1994
- (J) PUBLICATION DATE: 09-FEB-1995

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 38:

CACCCAAGAC AGCAGAAAGT

20

(2) INFORMATION FOR SEQ ID NO: 39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- 20 (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 38

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 39:

ACTTTCTGCT GTCTTGGGTG

20

(2) INFORMATION FOR SEQ ID NO: 40:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: Yes

- 75 -

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Vascular
Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

5 (H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 17)

(I) FILING DATE: 26-JUL-1994

(J) PUBLICATION DATE: 09-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 40:

CCATGGGTGC AGCCTGGGAC

20

10 (2) INFORMATION FOR SEQ ID NO: 41:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

15 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 40

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 41:

GTCGGCAGGCT GCACCCATGG

20

(2) INFORMATION FOR SEQ ID NO: 42:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

30 (D) OTHER INFORMATION: Antisense to Vascular
Endothelial Growth factor (VEGF)

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/04142 (SEQ ID NO: 17)

(I) FILING DATE: 26-JUL-1994

35 (J) PUBLICATION DATE: 09-FEB-1995

- 76 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 42:

CCATGGGTGC AGCCTGGGAC

20

(2) INFORMATION FOR SEQ ID NO: 43:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

10 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 42

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 43:

GTCCCAGGCT GCACCCATGG

20

15 (2) INFORMATION FOR SEQ ID NO: 44:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

20 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

25 (H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 1)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 44:

CCCTTCCTAC CGCGTGCGAC

20

30 (2) INFORMATION FOR SEQ ID NO: 45:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

- 77 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse

5 complement of SEQ ID NO: 44

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 45:

GTCGGACGCG GTAGGAAGGG

20

(2) INFORMATION FOR SEQ ID NO: 46:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

15 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 3)

(I) FILING DATE: 20-SEP-1994

20 (J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 46:

CCTCCGACCC ATCCACGTAG

20

(2) INFORMATION FOR SEQ ID NO: 47:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

30 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse

complement of SEQ ID NO: 46

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 47:

CTACGTGGAT GGGTCGGAGG

20

- 78 -

(2) INFORMATION FOR SEQ ID NO: 48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

10 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 5)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 48:

15 GTTGACGTCC TACGGAAACA

20

(2) INFORMATION FOR SEQ ID NO: 49:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse

25 complement of SEQ ID NO: 48

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 49:

TGTTCCGTA GGACGTCAAC

20

(2) INFORMATION FOR SEQ ID NO: 50:

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 17 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 79 -

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 8)

(I) FILING DATE: 20-SEP-1994

5 (J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 50:

CGCGTGCGAC CCTCTTG

17

(2) INFORMATION FOR SEQ ID NO: 51:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

15 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 51:

CAAGAGGGTC GCACGCG

17

20 (2) INFORMATION FOR SEQ ID NO: 52:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

30 (H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 9)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 52:

TCCTACCGCG TGCGACC

17

- 80 -

(2) INFORMATION FOR SEQ ID NO: 53:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 17 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 52

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 53:

GAGGGTCGCA CGCGGTA

17

(2) INFORMATION FOR SEQ ID NO: 54:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 17 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 10)

(I) FILING DATE: 20-SEP-1994

25 (J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 54:

TCCTTACCGCG TGCGGACC

17

(2) INFORMATION FOR SEQ ID NO: 55:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 17 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

35 (ix) FEATURE:

- 81 -

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 54

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 55:

GGTGGCACGC GGTAGGA

17

5 (2) INFORMATION FOR SEQ ID NO: 56:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 11)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 56:

CCTTCCTTACCGCGTGCG

17

20 (2) INFORMATION FOR SEQ ID NO: 57:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 56

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 57:

CGCACGCGGT AGGAAGG

17

(2) INFORMATION FOR SEQ ID NO: 58:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

- 82 -

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
5 (ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 12)
(I) FILING DATE: 20-SEP-1994
10 (J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 58:
GACCCTTCCT ACCGGT 17

(2) INFORMATION FOR SEQ ID NO: 59:
(i) SEQUENCE CHARACTERISTICS:
15 (A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
20 (ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 58
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 59:
ACGCCGTAGG AAGGGTC 17

25 (2) INFORMATION FOR SEQ ID NO: 60:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
35 (H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 13)

- 83 -

(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 60:
GGAGACCCCTT CCTACCG

17

5 (2) INFORMATION FOR SEQ ID NO: 61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
10 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 60
15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: BC-RC:
CGGTAGGAAG GGTCTCC

17

(2) INFORMATION FOR SEQ ID NO: 62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
20 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
25 (D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 14)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 62:
GCGGCGGCAG CGCGG

15

(2) INFORMATION FOR SEQ ID NO: 63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs

- 84 -

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
5 (ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 62
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 63:
CCGCGCTGCC GCCGC

15

10 (2) INFORMATION FOR SEQ ID NO: 64:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
15 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to bcl-2 mRNA
(x) PUBLICATION INFORMATION:
20 (H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 15)
(I) FILING DATE: 20-SEP-1994
(J) PUBLICATION DATE: 30-MAR-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 64:
CGGCGGGGCG ACGGA

15

25 (2) INFORMATION FOR SEQ ID NO: 65:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 64
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 65:

- 85 -

TCCGTCGCC CGCCG

15

(2) INFORMATION FOR SEQ ID NO: 66:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs

5 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

10 (D) OTHER INFORMATION: Antisense to bcl-2 mRNA

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 16)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 66:

CGGGAGCGCG GCGGGC

16

(2) INFORMATION FOR SEQ ID NO: 67:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 16 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 66

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 67:

GCCCGCCGCG CTCCCC

16

(2) INFORMATION FOR SEQ ID NO: 68:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

- 86 -

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl-2 mRNA; a.k.a. "BCL-2"

5 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/08350 (SEQ ID NO: 17)

(I) FILING DATE: 20-SEP-1994

(J) PUBLICATION DATE: 30-MAR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 68:

10 TCTCCCAGCG TGCGCCAT

18

(2) INFORMATION FOR SEQ ID NO: 69:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

15 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 68

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 69:

ATGGCGCACG CTGGGAGA

18

(2) INFORMATION FOR SEQ ID NO: 70:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 15 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

30 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to ζ -Protein Kinase C

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 93/20101 (SEQ ID NO: 14)

35 (I) FILING DATE: 02-APR-1993

- 87 -

(J) PUBLICATION DATE: 14-OCT-1993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 70:

GGTCCTGCTG GGCAT

15

(2) INFORMATION FOR SEQ ID NO: 71:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 70

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 71:

15 ATGCCAGCA GGACC

15

(2) INFORMATION FOR SEQ ID NO: 72:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

20 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to α -Protein Kinase C Gene; a.k.a. "ISIS 3521"

25 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/02069 (SEQ ID NO: 2)

(I) FILING DATE: 08-JUL-1994

(J) PUBLICATION DATE: 19-JAN-1995

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 72:

GTTCTCGCTG GTGAGTTCA

20

(2) INFORMATION FOR SEQ ID NO: 73:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs

- 88 -

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
5 (ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 72
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 73:
TGAAACTCAC CAGCGAGAAC 20

10 (2) INFORMATION FOR SEQ ID NO: 74:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
15 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to c-raf kinase Gene; a.k.a. "ISIS 5132"
20 (x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: US 5563255 (SEQ ID NO: 8)
(I) FILING DATE: 05-31-1994
(J) PUBLICATION DATE: 08-OCT-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 74:
25 TCCCGCCTGT GACATGCATT 20

(2) INFORMATION FOR SEQ ID NO: 75:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
30 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 74
35

- 89 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 75:

AATGCATGTC ACAGGCGGGA

20

(2) INFORMATION FOR SEQ ID NO: 76:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

10 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to bcl/abl mRNA

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 92/02641

(I) FILING DATE: 09-AUG-1991

15 (J) PUBLICATION DATE: 20-FEB-1992

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 76:

GGCGTTTGA ACTCTGCTT

19

(2) INFORMATION FOR SEQ ID NO: 77:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 19 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

25 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 76

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 77:

AAGCAGAGTT CAAAACGCC

19

30 (2) INFORMATION FOR SEQ ID NO: 78:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

- 90 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4
5 peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 1)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 78:
CCTCTCTGTT TAAAACTTA TCCAT 25

(2) INFORMATION FOR SEQ ID NO: 79:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 25 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

20 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 78

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 79:
ATGGATAAAG TTTTAAACAG AGAGG 25

(2) INFORMATION FOR SEQ ID NO: 80:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

30 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

35 (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 2)

- 91 -

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 80:

TTCATATCCT GAGTCATGTC G

21

5 (2) INFORMATION FOR SEQ ID NO: 81:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 80

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 81:

CGACATGACT CAGGATATGA A

21

(2) INFORMATION FOR SEQ ID NO: 82:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Antisense to beta/A4
peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 3)

(I) FILING DATE: 28-SEP-1994

30 (J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 82:

GTCCCGAGCGC TACGACGGGC CAAA

24

(2) INFORMATION FOR SEQ ID NO: 83:

(i) SEQUENCE CHARACTERISTICS:

- 92 -

- (A) LENGTH: 24 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

5 (iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 82

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 83:

10 TTTGGCCCGT CGTAGCGCTG GGAC 24

(2) INFORMATION FOR SEQ ID NO: 84:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 base pairs
- (B) TYPE: Nucleic Acid

15 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to beta/A4 peptide

20 (x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 4)
- (I) FILING DATE: 28-SEP-1994
- (J) PUBLICATION DATE: 06-APR-1995

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 84:
GTCCCAGCGC TAC 13

(2) INFORMATION FOR SEQ ID NO: 85:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 13 base pairs
- (B) TYPE: Nucleic Acid

30 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse

- 93 -

complement of SEQ ID NO: 84

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 85:

GTAGCGCTGG GAC

13

(2) INFORMATION FOR SEQ ID NO: 86:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 5)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 86:

TACGACGGGC CAAA

14

20 (2) INFORMATION FOR SEQ ID NO: 87:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 86

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 87:

TTTGGCCCGT CGTA

14

(2) INFORMATION FOR SEQ ID NO: 88:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs

- 94 -

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

5 (ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 6)

10 (I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 88:

GTCCCCAGCGC TACGACGGGC C

21

(2) INFORMATION FOR SEQ ID NO: 89:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

20 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 88

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 89:

25 GGCCCGTCGT AGCGCTGGGA C

21

(2) INFORMATION FOR SEQ ID NO: 90:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

35

- 95 -

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 7)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 90:

GTCCCAAGCGC TACGACGG

18

(2) INFORMATION FOR SEQ ID NO: 91:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

10 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

15 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 90

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 91:

CCGTCGTAGC GCTGGGAC

18

(2) INFORMATION FOR SEQ ID NO: 92:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

25 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

30 (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 8)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 92:

GTCCCAAGCGC TACGA

15

- 96 -

(2) INFORMATION FOR SEQ ID NO: 93:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 92

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 93:

TCGTAGCGCT GGGAC

15

(2) INFORMATION FOR SEQ ID NO: 94:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 21 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

(x) PUBLICATION INFORMATION:

25 (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 9)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 94:

CCAGCGCTAC GACGGGCCAA A

21

(2) INFORMATION FOR SEQ ID NO: 95:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: No

- 97 -

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 94

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 95:

5 TTTGGCCCGT CGTAGCGCTG G

21

(2) INFORMATION FOR SEQ ID NO: 96:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

10 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4 peptide

15 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 10)

(I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 96:

GCGCTACGAC GGGCCAAA

18

(2) INFORMATION FOR SEQ ID NO: 97:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

30 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 96

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 97:

TTTGGCCCGT CGTAGCGC

18

(2) INFORMATION FOR SEQ ID NO: 98:

- 98 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- 5 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta/A4
peptide

10 (x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 11)
- (I) FILING DATE: 28-SEP-1994
- (J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 98:

15 CTACGACGGG CCAAA

15

(2) INFORMATION FOR SEQ ID NO: 99:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 15 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
25 complement of SEQ ID NO: 98

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 99:

TTTGGCCCGT CGTAG

15

(2) INFORMATION FOR SEQ ID NO: 100:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 24 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 99 -

(D) OTHER INFORMATION: Antisense to beta/A4
peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/09236 (SEQ ID NO: 15)

5 (I) FILING DATE: 28-SEP-1994

(J) PUBLICATION DATE: 06-APR-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 100:

AAACCGGGCA GCATCGCGAC CCTG

24

(2) INFORMATION FOR SEQ ID NO: 101:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 24 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

15 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 100

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 101:

20 CAGGGTCGCG ATGCTGCCCG GTTT

24

(2) INFORMATION FOR SEQ ID NO: 102:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

25 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to beta-globin;
a.k.a. "5'ss"

30 (x) PUBLICATION INFORMATION:

(A) AUTHORS: Sierakowska, H., et al.

(B) TITLE: Repair of thalassemic human b-globin in
mammalian cells by antisense oligonucleotides

35 (C) JOURNAL: The Proceedings of the National

- 100 -

Academy of Sciences (U.S.A.)

(D) VOLUME: 93
(F) PAGES: 12840-12844
(G) DATE: 12-NOV-1996

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 102:
GCUAUUACCU UAACCCAG

18

(2) INFORMATION FOR SEQ ID NO: 103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 103:
CTGGGTAAAG GTAATAGC

18

(2) INFORMATION FOR SEQ ID NO: 104:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
25 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to beta-globin;
a.k.a. "3'ss"
(x) PUBLICATION INFORMATION:
30 (A) AUTHORS: Sierakowska, H., et al.
(B) TITLE: Repair of thalassemic human b-globin in
mammalian cells by antisense oligonucleotides
(C) JOURNAL: The Proceedings of the National
Academy of Sciences (U.S.A.)
35 (D) VOLUME: 93

- 101 -

(F) PAGES: 12840-12844

(G) DATE: 12-NOV-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 104:

CAUUAUUGCC CUGAAAG

17

5 (2) INFORMATION FOR SEQ ID NO: 105:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 104

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 105:

CTTTCAAGGGC AATAATG

17

(2) INFORMATION FOR SEQ ID NO: 106:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Antisense to Multi-drug
Resistance-1 (MDR-1) gene

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO: 1)

(I) FILING DATE: 18-JUL-1995

30 (J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 106:

TGTGCTCTTC CCACAGCCAC TG

22

(2) INFORMATION FOR SEQ ID NO: 107:

(i) SEQUENCE CHARACTERISTICS:

- 102 -

(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
5 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 106
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 107:
10 CAGTGGCTGT GGGAAAGAGCA CA

22

(2) INFORMATION FOR SEQ ID NO: 108:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
15 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug
20 Resistance-1 (MDR-1) gene
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO: 2)
(I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 108:
TGTGCTCTTC CCACAGCCAC

20

(2) INFORMATION FOR SEQ ID NO: 109:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
30 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse

- 103 -

complement of SEQ ID NO: 108

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 109:

GTGGCTGTGG GAAGAGCACA

20

(2) INFORMATION FOR SEQ ID NO: 110:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to Multi-drug
Resistance-1 (MDR-1) gene

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO: 3)

(I) FILING DATE: 18-JUL-1995

(J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 110:

GTGCTTCC CACAGCCACT

20

20 (2) INFORMATION FOR SEQ ID NO: 111:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 110

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 111:

AGTGGCTGTG GGAAGAGCAC

20

(2) INFORMATION FOR SEQ ID NO: 112:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

- 104 -

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
5 (ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Multi-drug
Resistance-1 (MDR-1) gene
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02556 (SEQ ID NO: 4)
10 (I) FILING DATE: 18-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 112:
TGCTCTTCCC ACAGCCACTG 20

(2) INFORMATION FOR SEQ ID NO: 113:
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
20 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 112
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 113:
25 CAGTGGCTGT GGGAAAGAGCA 20

(2) INFORMATION FOR SEQ ID NO: 114:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
30 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to malarial
agents; a.k.a. "PSI"
35

- 105 -

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 93/13740

(I) FILING DATE: 31-DEC-1991

(J) PUBLICATION DATE: 22-JUL-1993

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 114:

TAAAAAGAAT ATGATCTTCA T

21

(2) INFORMATION FOR SEQ ID NO: 115:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

10 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

15 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 114

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 115:

ATGAAGATCA TATTCTTTT A

21

(2) INFORMATION FOR SEQ ID NO: 116:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 18 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

25 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "PSII"

(x) PUBLICATION INFORMATION:

30 (H) DOCUMENT NUMBER: WO 93/13740 (SEQ ID NO: PSII)

(I) FILING DATE: 31-DEC-1991

(J) PUBLICATION DATE: 22-JUL-1993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 116:

AGCAACTGAG CCACCTGA

18

- 106 -

(2) INFORMATION FOR SEQ ID NO: 117:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 18 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 116

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 117:

TCAGGTGGCT CAGTTGCT

18

(2) INFORMATION FOR SEQ ID NO: 118:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 21 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "PSIII"

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 93/13740

25 (I) FILING DATE: 31-DEC-1991

(J) PUBLICATION DATE: 22-JUL-1993

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 118:

GTCGCAGACT TGTTCCATCA T

21

(2) INFORMATION FOR SEQ ID NO: 119:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: No

- 107 -

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 118

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 119:

5 ATGATGGAAC AAGTCTGCGA C

21

(2) INFORMATION FOR SEQ ID NO: 120:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

10 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to malarial agents; a.k.a. "RI"

15 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 93/13740

(I) FILING DATE: 31-DEC-1991

(J) PUBLICATION DATE: 22-JUL-1993

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 120:

CTTGGCAGCT GCGCGTGACA T

21

(2) INFORMATION FOR SEQ ID NO: 121:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

30 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 120

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 121:

ATGTCACGCG CAGCTGCCAA G

21

(2) INFORMATION FOR SEQ ID NO: 122:

- 108 -

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- 5 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to scistosome worms

10 (x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/33759 (SEQ ID NO: 1)
- (I) FILING DATE: 30-MAY-1995
- (J) PUBLICATION DATE: 14-DEC-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 122:

15 GCCATAGGGG GCAGGGAAGG C

21

(2) INFORMATION FOR SEQ ID NO: 123:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 122

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 123:

GCCTTCCCTG CCCCCCTATGG C

21

(2) INFORMATION FOR SEQ ID NO: 124:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 12 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 109 -

(D) OTHER INFORMATION: Antisense to HTLV-III

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: A)

(I) FILING DATE: 22-MAY-1987

5 (J) PUBLICATION DATE: 03-DEC-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 124:

CTGCTAGAGA TT

12

(2) INFORMATION FOR SEQ ID NO: 125:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 12 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

15 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 124

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 125:

AATCTCTAGC AG

12

20 (2) INFORMATION FOR SEQ ID NO: 126:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HTLV-III

(x) PUBLICATION INFORMATION:

30 (H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: B)

(I) FILING DATE: 22-MAY-1987

(J) PUBLICATION DATE: 03-DEC-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 126:

CTGCTAGAGA TTTTCCACAC

20

- 110 -

(2) INFORMATION FOR SEQ ID NO: 127:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 126

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 127:

GTGTGGAAAA TCTCTAGCAG

20

(2) INFORMATION FOR SEQ ID NO: 128:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 25 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to HTLV-III

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: C)

(I) FILING DATE: 22-MAY-1987

25 (J) PUBLICATION DATE: 03-DEC-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 128:

TTCAAGTCCC TGTTGGGGCG CCAAA

25

(2) INFORMATION FOR SEQ ID NO: 129:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 25 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

35 (ix) FEATURE:

- 111 -

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 128

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 129:
TTTGGCGCCC GAACAGGGAC TTGAA

25

5 (2) INFORMATION FOR SEQ ID NO: 130:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- 10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HTLV-III

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: D)

(I) FILING DATE: 22-MAY-1987

(J) PUBLICATION DATE: 03-DEC-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 130:

GCCTACTCAC CAGTCGCCGC

20

20 (2) INFORMATION FOR SEQ ID NO: 131:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 130

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 131:

GCGGCGACTG GTGAGTACGC

20

(2) INFORMATION FOR SEQ ID NO: 132:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 base pairs

- 112 -

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- (iv) ANTI-SENSE: Yes
- 5 (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to HTLV-III
 - (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: E)
 - (I) FILING DATE: 22-MAY-1987
 - 10 (J) PUBLICATION DATE: 03-DEC-1987
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 132:
CTGCTAGAGA TTAA 14
- (2) INFORMATION FOR SEQ ID NO: 133:
 - (i) SEQUENCE CHARACTERISTICS:
 - 15 (A) LENGTH: 14 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
 - (iv) ANTI-SENSE: No
 - 20 (ix) FEATURE:
 - (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 132
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 133:
TTAATCTCTA GCAG 14
 - 25 (2) INFORMATION FOR SEQ ID NO: 134:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - 30 (D) TOPOLOGY: Linear
 - (iv) ANTI-SENSE: Yes
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to HTLV-III
 - (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 87/07300 (SEQ ID NO: F)

- 113 -

(I) FILING DATE: 22-MAY-1987

(J) PUBLICATION DATE: 03-DEC-1987

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 134:

ACACCCAATT CTGAAAATGG

20

5 (2) INFORMATION FOR SEQ ID NO: 135:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 134

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 135:

CCATTTTCAG AATTGGGTGT

20

(2) INFORMATION FOR SEQ ID NO: 136:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(A) AUTHORS: Agrawal, Sudhir Tang, Jin Yan

(B) TITLE: GEM 91-An Antisense Oligonucleotide
Phosphorothioate as a Therapeutic Agent for AIDS

30 (C) JOURNAL: Antisense Research and Development

(D) VOLUME: 2

(E) ISSUE: 6

(F) PAGES: 261-266

(G) DATE: Winter-1992

35 (x) PUBLICATION INFORMATION:

- 114 -

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 1)

(I) FILING DATE: 04-OCT-1993

(J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 136:

5 CTCTCGCACC CATCTCTCTC CTTCT

25

(2) INFORMATION FOR SEQ ID NO: 137:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

(B) TYPE: Nucleic Acid

10 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
15 complement of SEQ ID NO: 136

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 137:

AGAAGGAGAG AGATGGGTGC GAGAG

25

(2) INFORMATION FOR SEQ ID NO: 138:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

25 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 2)

(I) FILING DATE: 04-OCT-1993

30 (J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 138:

CTCTCGCACC CATCTCTCTC CTTCTA

26

(2) INFORMATION FOR SEQ ID NO: 139:

(i) SEQUENCE CHARACTERISTICS:

- 115 -

- (A) LENGTH: 26 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- 5 (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 138
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 139:
10 TAGAAGGAGA GAGATGGGTG CGAGAG 26

- (2) INFORMATION FOR SEQ ID NO: 140:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: Nucleic Acid
 - 15 (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
 - (iv) ANTI-SENSE: Yes
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to HIV-1
 - 20 (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 3)
 - (I) FILING DATE: 04-OCT-1993
 - (J) PUBLICATION DATE: 14-APR-1994
 - (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 140:
25 GCTCTCGCAC CCATCTCTCT CCTTCT 26

- (2) INFORMATION FOR SEQ ID NO: 141:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: Nucleic Acid
 - 30 (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
 - (iv) ANTI-SENSE: No
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 140

- 116 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 141:
AGAAGGAGAG AGATGGGTGC GAGAGC

26

(2) INFORMATION FOR SEQ ID NO: 142:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

10 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 4)

(I) FILING DATE: 04-OCT-1993

15 (J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 142:

GCTCTCGCAC CCATCTCTCT CCTTCTA

27

(2) INFORMATION FOR SEQ ID NO: 143:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 27 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

25 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 142

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 143:

TAGAAGGAGA GAGATGGGTG CGAGAGC

27

30 (2) INFORMATION FOR SEQ ID NO: 144:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 28 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

- 117 -

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
5 (x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 5)
(I) FILING DATE: 04-OCT-1993
(J) PUBLICATION DATE: 14-APR-1994
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 144:
10 GCTCTCGCAC CCATCTCT CCTTCTAG 28

(2) INFORMATION FOR SEQ ID NO: 145:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: Nucleic Acid
15 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse
20 complement of SEQ ID NO: 144
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 145:
CTAGAAGGAG AGAGATGGGT GCGAGAGC 28

(2) INFORMATION FOR SEQ ID NO: 146:
(i) SEQUENCE CHARACTERISTICS:
25 (A) LENGTH: 28 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
30 (ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV-1
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 6)
(I) FILING DATE: 04-OCT-1993
35 (J) PUBLICATION DATE: 14-APR-1994

- 118 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 146:

CGCTCTCGCA CCCATCTCTC TCCTTCTA

28

(2) INFORMATION FOR SEQ ID NO: 147:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 28 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

10 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 146

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 147:

TAGAAGGAGA GAGATGGGTG CGAGAGCG

28

15 (2) INFORMATION FOR SEQ ID NO: 148:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

20 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

25 (H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 7)

(I) FILING DATE: 04-OCT-1993

(J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 148:

CGCTCTCGCA CCCATCTCTC TCCTTCTAG

29

30 (2) INFORMATION FOR SEQ ID NO: 149:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

- 119 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse

5 complement of SEQ ID NO: 148

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 149:

CTAGAAGGAG AGAGATGGGT GCGAGAGCG

29

(2) INFORMATION FOR SEQ ID NO: 150:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 30 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

15 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 8)

(I) FILING DATE: 04-OCT-1993

20 (J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 150:

CGCTCTCGCA CCCATCTCTC TCCTTCTAGC

30

(2) INFORMATION FOR SEQ ID NO: 151:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 30 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

30 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse

complement of SEQ ID NO: 150

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 151:

GCTAGAAGGA GAGAGATGGG TGCGAGAGCG

30

- 120 -

(2) INFORMATION FOR SEQ ID NO: 152:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1

10 (x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 9)
- (I) FILING DATE: 04-OCT-1993
- (J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 152:

15 ACGCTCTCGC ACCCATCTCT CTCCTTCTAG

30

(2) INFORMATION FOR SEQ ID NO: 153:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
25 complement of SEQ ID NO: 152

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 153:

CTAGAAGGAG AGAGATGGGT GCGAGAGCGT

30

(2) INFORMATION FOR SEQ ID NO: 154

(i) SEQUENCE CHARACTERISTICS:

30

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 121 -

(D) OTHER INFORMATION: Antisense to HIV-1

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 94/08004 (SEQ ID NO: 10)

(I) FILING DATE: 04-OCT-1993

5 (J) PUBLICATION DATE: 14-APR-1994

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 154:

CTCGCACCCA TCTCTCTCCT

20

(2) INFORMATION FOR SEQ ID NO: 155:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

15 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 154

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 155:

AGGAGAGAGA TGGGTGCGAG

20

20 (2) INFORMATION FOR SEQ ID NO: 156:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV-1; a.k.a.
"AR 177"

30 (x) PUBLICATION INFORMATION:

(A) AUTHORS: Bishop, J.S., et al.

(B) TITLE: Intramolecular G-quartet Motifs Confer
Nuclease Resistance to a Potent Anti-HIV
Oligonucleotide

35 (C) JOURNAL: The Journal of Biological Chemistry

- 122 -

- (D) VOLUME: 271
- (E) ISSUE: 10
- (F) PAGES: 5698-5703
- (G) DATE: 08-MAR-1996

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 156:
GTGGTGGGTG GGTGGGT

17

(2) INFORMATION FOR SEQ ID NO: 157:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 17 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: No
(ix) FEATURE:
15 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 156
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 157:
ACCCACCCAC CCACCAC

17

(2) INFORMATION FOR SEQ ID NO: 158:

20 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 26 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

25 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 158:
GCCTATTCTG CTATGTCGAC ACCCAA

26

30 (2) INFORMATION FOR SEQ ID NO: 159:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single

- 123 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 158

5 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03407 (SEQ ID NO: 94)

(I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 159:
UUGGGUGUCG ACAUAGCAGA AUAGGC 26

(2) INFORMATION FOR SEQ ID NO: 160:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

20 (D) OTHER INFORMATION: Antisense to HIV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 160:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 161:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

30 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 160

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03407

35 (I) FILING DATE: 19-JUL-1994

- 124 -

(J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 161:

CCCGAGGGGA CCCGACAGGC CCGAAG 26

(2) INFORMATION FOR SEQ ID NO: 162:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03407

15 (I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 162:

CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 163:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

25 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 162

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 163:

30 CCCGAGGGGA CCCGACAGGC CCGAAG 26

(2) INFORMATION FOR SEQ ID NO: 164:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

- 125 -

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
5 (D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 3)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 164:
GCTGGTGATC CTTTCCATCC CTGTGG 26

(2) INFORMATION FOR SEQ ID NO: 165:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
15 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
20 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 164
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 165:
CCACAGGGAT GGAAAGGATC ACCAGC 26

(2) INFORMATION FOR SEQ ID NO: 166:
25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
30 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 5)
35 (I) FILING DATE: 14-JUL-1995

- 126 -

(J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 166:

CTACTACTCC TTGACTTTGG GGATTG

26

(2) INFORMATION FOR SEQ ID NO: 167:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 167:

15 CAATCCCCAA AGTCAAGGAG TAGTAG

26

(2) INFORMATION FOR SEQ ID NO: 168:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: Nucleic Acid

20 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV

25 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 6)

(I) FILING DATE: 14-JUL-1995

(J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 168:

30 CCTCTGTTAG TAACATATCC TGCTTTCC

29

(2) INFORMATION FOR SEQ ID NO: 169:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 29 base pairs

(B) TYPE: Nucleic Acid

- 127 -

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
5 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 168
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 169:
GGAAAAGCAG GATATGTTAC TAACAGAGG

29

(2) INFORMATION FOR SEQ ID NO: 170:
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
15 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 8)
20 (I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 170:
GGTTGCTTCC TTCCTCTCTG GTACCC

26

(2) INFORMATION FOR SEQ ID NO: 171:
25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
30 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 170
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 171:
35 GGGTACCAAGA GAGGAAGGAA GCAACC

26

- 128 -

(2) INFORMATION FOR SEQ ID NO: 172:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

- (D) OTHER INFORMATION: Antisense to HIV

10 (x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 10)
- (I) FILING DATE: 14-JUL-1995
- (J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 172:

| | | |
|----|------------------------|----|
| 15 | CTAGCAGTGG CGCCCGAAC A | 30 |
| | TTGGGGCGCC | 41 |

(2) INFORMATION FOR SEQ ID NO: 173:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 41 base pairs
- 20 (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 172

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 173:

| | |
|-----------------------|----|
| TGGCGCCCGA ACAGGCGAAC | 30 |
| GCCACTGCTA G | 41 |

30 (2) INFORMATION FOR SEQ ID NO: 174:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- 35 (D) TOPOLOGY: Linear

- 129 -

(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
5 (H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 22)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 174:
CATCACCTGC CATCTGTTT CCATAATCCC

30

10 (2) INFORMATION FOR SEQ ID NO: 175:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
15 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 174
20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 175:
GGGATTATGG AAAACAGATG GCAGGTGATG

30

(2) INFORMATION FOR SEQ ID NO: 176:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
25 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
30 (D) OTHER INFORMATION: Antisense to HIV
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 23)
(I) FILING DATE: 14-JUL-1995
(J) PUBLICATION DATE: 01-FEB-1996
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 176:

- 130 -

CCTGTCTACT TGCCACACAA TCATCACCTG C

31

(2) INFORMATION FOR SEQ ID NO: 177:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 31 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(10) (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 176

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 177:

GCAGGTGATG ATTGTGTGGC AAGTAGACAG G

31

(2) INFORMATION FOR SEQ ID NO: 178:

(15) (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 96/02557 (SEQ ID NO: 25)

(25) (I) FILING DATE: 14-JUL-1995

(J) PUBLICATION DATE: 01-FEB-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 178:

ACTATTGCTA TTATTATTGC TACTACTAAT

30

(2) INFORMATION FOR SEQ ID NO: 179:

(30) (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

- 131 -

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 178

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 179:
ATTAGTAGTA GCAATAATAA TAGCAATAGT 30

(2) INFORMATION FOR SEQ ID NO: 180:

(i) SEQUENCE CHARACTERISTICS:

10 (A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

15 (D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 1)

(I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 180:
CTTCGGGCCT GTCGGGTCCC CTCGGG 26

(2) INFORMATION FOR SEQ ID NO: 181:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 26 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

30 (D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 2)

(I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: HIV:

- 132 -

CUUCGGGCCU GUCGGGUCC CUCGGG

30

(2) INFORMATION FOR SEQ ID NO: 182:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

5 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

10 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NOS: 180 and 181

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 182:

CCCGAGGGGA CCCGACAGGC CCCGAAAG

26

(2) INFORMATION FOR SEQ ID NO: 183:

15 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

20 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 3)

25 (I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 183:

GCCTGTCGGG TCCC

14

(2) INFORMATION FOR SEQ ID NO: 184:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

- 133 -

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

5 (H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 4)

(I) FILING DATE: 19-JUL-1994

(J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 184:

GCCUGUCGGG UCCC

14

10 (2) INFORMATION FOR SEQ ID NO: 185:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

15 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NOS: 183 and 184

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 185:

GGGACCCGAC AGGC

14

(2) INFORMATION FOR SEQ ID NO: 186:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 26 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

30 (D) OTHER INFORMATION: Antisense to HIV

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 186:

CTTCGGGCCT GTCGGGTCCC CTCGGG

26

(2) INFORMATION FOR SEQ ID NO: 187:

(i) SEQUENCE CHARACTERISTICS:

- 134 -

- (A) LENGTH: 26 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

5 (iv) ANTI-SENSE: No

(ix) FEATURE:

- (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 186

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 5)
- (I) FILING DATE: 19-JUL-1994
- (J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 187:
CCCGAGGGGA CCCGACAGGC CCGAAG 26

15 (2) INFORMATION FOR SEQ ID NO: 188:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (iv) ANTI-SENSE: Yes
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to HIV

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/03406 (SEQ ID NO: 6)
- (I) FILING DATE: 19-JUL-1994
- (J) PUBLICATION DATE: 02-FEB-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 188:
GCTGGTGATC CTTTCCATCC CTGTGG 26

30 (2) INFORMATION FOR SEQ ID NO: 189:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 26 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear

- 135 -

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 188

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 189:

CCACAGGGAT GGAAAGGATC ACCAGC

26

(2) INFORMATION FOR SEQ ID NO: 190:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 base pairs

10 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

15 (D) OTHER INFORMATION: Antisense to HIV; a.k.a.
"ISIS 5320"

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: US 5523389

(I) FILING DATE: 28-SEP-1994

20 (J) PUBLICATION DATE: 04-JUN-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 190:

TTGGGGTT

8

(2) INFORMATION FOR SEQ ID NO: 191:

(i) SEQUENCE CHARACTERISTICS:

25 (A) LENGTH: 8 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

30 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 190

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 191:

AACCCCAA

8

- 136 -

(2) INFORMATION FOR SEQ ID NO: 192:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

10 (D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 1)
- (I) FILING DATE: 29-APR-1991
- (J) PUBLICATION DATE: 14-NOV-1991

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 192:

CATTCAAATG GTTTGCCTGC

20

(2) INFORMATION FOR SEQ ID NO: 193:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- 20 (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

25 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 192

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 193:

GCAGGCAAAC CATTGAAATG

20

(2) INFORMATION FOR SEQ ID NO: 194:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: Yes

- 137 -

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

5 (H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 2)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 194:

GCAGGCAAAC CATTGAAATG

20

10 (2) INFORMATION FOR SEQ ID NO: 195:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

15 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 194

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 195:

CATTCAAATG GTTTGCCTGC

20

(2) INFORMATION FOR SEQ ID NO: 196:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

30 (D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 3)

(I) FILING DATE: 29-APR-1991

35 (J) PUBLICATION DATE: 14-NOV-1991

- 138 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 196:

CCATAATCCC CTGCTTCTGC

20

(2) INFORMATION FOR SEQ ID NO: 197:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

10 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 196

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 197:

GCAGAACGAG GGGATTATGG

20

15 (2) INFORMATION FOR SEQ ID NO: 198:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

20 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza virus

25 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 4)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 198:

30 GCAGAACGAG GGGATTATGG

20

(2) INFORMATION FOR SEQ ID NO: 199:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

- 139 -

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
5 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 198
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 199:
CCATAATCCC CTGCTTCTGC 20

(2) INFORMATION FOR SEQ ID NO: 200:
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
15 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to influenza virus
(x) PUBLICATION INFORMATION:
20 (H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 5)
(I) FILING DATE: 29-APR-1991
(J) PUBLICATION DATE: 14-NOV-1991
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 200:
GCAGAAGCAG AGGATTATGG 20

25 (2) INFORMATION FOR SEQ ID NO: 201:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 200
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 201:

- 140 -

CCATAATCCT CTGCTTCTGC

20

(2) INFORMATION FOR SEQ ID NO: 202:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- 5 (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

10 (D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 6)
- (I) FILING DATE: 29-APR-1991

15 (J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 202:

GCATAAGCAG AGGATCATGG

20

(2) INFORMATION FOR SEQ ID NO: 203:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 20 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

25 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 202

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 203:

CCATGATCCT CTGCTTATGC

20

30 (2) INFORMATION FOR SEQ ID NO: 204:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single

- 141 -

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza
5 virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 7)

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 204:
GGCAAGCTTT ATTGAGGCTT 20

(2) INFORMATION FOR SEQ ID NO: 205:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

20 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 204

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 205:
AAGCCTCAAT AAAGCTTGCC 20

(2) INFORMATION FOR SEQ ID NO: 206:

25 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

30 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

35 (H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 8)

- 142 -

(I) FILING DATE: 29-APR-1991

(J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 206:

ATCTTCATCA TCTGAGAGAT

20

5 (2) INFORMATION FOR SEQ ID NO: 207:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 206

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 207:

ATCTCTCAGA TGATGAAGAT

20

(2) INFORMATION FOR SEQ ID NO: 208:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Antisense to influenza
virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 91/16902 (SEQ ID NO: 9)

(I) FILING DATE: 29-APR-1991

30 (J) PUBLICATION DATE: 14-NOV-1991

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 208:

CGTAAGCAAC AGTAGTCCTA

20

(2) INFORMATION FOR SEQ ID NO: 209:

(i) SEQUENCE CHARACTERISTICS:

- 143 -

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear
- 5 (iv) ANTI-SENSE: No
- (ix) FEATURE:
 - (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 208
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 209:
10 TAGGACTACT GTTGCTTACG 20

- (2) INFORMATION FOR SEQ ID NO: 210:
 - (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 20 base pairs
 - (B) TYPE: Nucleic Acid
 - 15 (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
 - (iv) ANTI-SENSE: Yes
 - (ix) FEATURE:
 - (D) OTHER INFORMATION: Antisense to
20 cytomagalovirus intron-exon boundary of genes UL36 and UL37; a.k.a. "UL36ANTI" and "GEM 132"
 - (x) PUBLICATION INFORMATION:
 - (A) AUTHORS: Pari, G.S., et al.
 - (B) TITLE: Potent Antiviral Activity of an
25 Antisense Oligonucleotide Complementary to the
Intron-Exon Boundary of Human Cytomegalovirus
Genes UL36 and UL37
 - (C) JOURNAL: Antimicrobial Agents and Chemotherapy
 - (D) VOLUME: 39
 - 30 (E) ISSUE: 5
 - (F) PAGES: 1157-1161
 - (G) DATE: MAY-1995
 - (x) PUBLICATION INFORMATION:
 - (H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 1)
 - (I) FILING DATE: 19-MAY-1995
 - (J) PUBLICATION DATE: 30-NOV-1995

- 144 -

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 210:

TGGGGCTTAC CTTGCGAAC

20

(2) INFORMATION FOR SEQ ID NO: 211:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

10 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 210

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 211:

TGTCGCAAG GTAAGCCCCA

20

15 (2) INFORMATION FOR SEQ ID NO: 212:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single

20 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to
cytomegalovirus

25 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 2)

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 212:

30 GACGTGGGGC TTACCTTGCG

20

(2) INFORMATION FOR SEQ ID NO: 213:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid

- 145 -

(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
5 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 212
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 213:
CGCAAGGTAA GCCCCACGTC 20

(2) INFORMATION FOR SEQ ID NO: 214:
10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
15 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to cytomegalovirus
(x) PUBLICATION INFORMATION:
20 (H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 3)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 214:
TCTTCAACGA CGTGGGGCTT 20

25 (2) INFORMATION FOR SEQ ID NO: 215:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 214
35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 215:

- 146 -

AAGCCCCACG TCGTTGAAGA

20

(2) INFORMATION FOR SEQ ID NO: 216:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- 5 (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

10 (D) OTHER INFORMATION: Antisense to
cytomaglovirus

(x) PUBLICATION INFORMATION:

- (H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 4)
- (I) FILING DATE: 19-MAY-1995

15 (J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 216:

GACGCGTGGC ATGCTTGGTG T

21

(2) INFORMATION FOR SEQ ID NO: 217:

(i) SEQUENCE CHARACTERISTICS:

20 (A) LENGTH: 21 base pairs

- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

25 (ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 216

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 217:

ACACCAAGCA TGCCACGCGT C

21

30 (2) INFORMATION FOR SEQ ID NO: 218:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single

- 147 -

(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to
5 cytomagalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 5)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 218:
AGGTTGGGGT CGACGCGTGG C 21

(2) INFORMATION FOR SEQ ID NO: 219:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
15 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
20 (D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 218
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 219:
GCCACGCGTC GACCCCAACC T 21

(2) INFORMATION FOR SEQ ID NO: 220:
25 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
30 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to
cytomagalovirus
(x) PUBLICATION INFORMATION:
35 (H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 6)

- 148 -

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 220:

GGCTGAGCGG TCATCCTCGG A

21

5 (2) INFORMATION FOR SEQ ID NO: 221:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

10 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 220

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 221:

TCCGAGGATG ACCGCTCAGC C

21

(2) INFORMATION FOR SEQ ID NO: 222:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

20 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

25 (D) OTHER INFORMATION: Antisense to
cytomaglovirus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 7)

(I) FILING DATE: 19-MAY-1995

30 (J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 222:

CGGGACTCAC CGTCGTTCTG

20

(2) INFORMATION FOR SEQ ID NO: 223:

(i) SEQUENCE CHARACTERISTICS:

- 149 -

(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
5 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 222
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 223:
10 CAGAACGACG GTGAGTCCCG

20

(2) INFORMATION FOR SEQ ID NO: 224:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20
(B) TYPE: Nucleic Acid
15 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to
20 cytomegalovirus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 8)
(I) FILING DATE: 19-MAY-1995
(J) PUBLICATION DATE: 30-NOV-1995
25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 224:
GGAGGGAGAGC CTACAGACGG

20

(2) INFORMATION FOR SEQ ID NO: 225:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
30 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
35 (D) OTHER INFORMATION: Full-length reverse

- 150 -

complement of SEQ ID NO: 224

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 225:

CCGTCTGTAG GCTCTCCTCC

20

(2) INFORMATION FOR SEQ ID NO: 226:

5 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

10 (iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to
cytomaglovirus

(x) PUBLICATION INFORMATION:

15 (H) DOCUMENT NUMBER: WO 95/32213 (SEQ ID NO: 9)

(I) FILING DATE: 19-MAY-1995

(J) PUBLICATION DATE: 30-NOV-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 226:

AGTAACGCAC CGTCGGTGCC

20

20 (2) INFORMATION FOR SEQ ID NO: 227:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

25 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 226

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 227:

GGCACCGACG GTGCGTTACT

20

(2) INFORMATION FOR SEQ ID NO: 228:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 25 base pairs

- 151 -

(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
5 (ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Epstein-Barr
Virus
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO: 1)
10 (I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 228:
TTTGGGTCCA TCATCTTCAG CAAAG 25

(2) INFORMATION FOR SEQ ID NO: 229:
15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
20 (iv) ANTI-SENSE: No
(ix) FEATURE:
(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 228
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 229:
25 CTTTGCTGAA GATGATGGAC CCAAA 25

(2) INFORMATION FOR SEQ ID NO: 230:
30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Epstein-Barr
35 Virus

- 152 -

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO: 2)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995

5 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 230:
CATCATCTTC AGCAAAGATA 20

(2) INFORMATION FOR SEQ ID NO: 231:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
10 (B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
(iv) ANTI-SENSE: No
(ix) FEATURE:
15 (D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 230
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 231:
TATCTTGCT GAAGATGATG 20

(2) INFORMATION FOR SEQ ID NO: 232:
20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear
25 (iv) ANTI-SENSE: Yes
(ix) FEATURE:
(D) OTHER INFORMATION: Antisense to Epstein-Barr Virus
(x) PUBLICATION INFORMATION:
30 (H) DOCUMENT NUMBER: WO 95/22554 (SEQ ID NO: 3)
(I) FILING DATE: 17-FEB-1995
(J) PUBLICATION DATE: 24-AUG-1995
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 232:
TCAGAAAGTCG AGTTTGGGTC 20

- 153 -

(2) INFORMATION FOR SEQ ID NO: 233:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 5 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
10 complement of SEQ ID NO: 232

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 233:

GACCCAAACT CGACTTCTGA

20

(2) INFORMATION FOR SEQ ID NO: 234:

(i) SEQUENCE CHARACTERISTICS:

- 15 (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

20 (ix) FEATURE:

(D) OTHER INFORMATION: Antisense to
Respiratory Syncytial Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO: 1)

25 (I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 234:

ACGCGAAAAA ATGCGTACAA

20

(2) INFORMATION FOR SEQ ID NO: 235:

30 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

35 (iv) ANTI-SENSE: No

- 154 -

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse complement of SEQ ID NO: 234

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 235:

5 TTGTACGCAT TTTTCGCGT

20

(2) INFORMATION FOR SEQ ID NO: 236:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

10 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to

15 Respiratory Syncytial Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO: 2)

(I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

20 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 236:

TAAACCAAAA AAATGGGGCA

20

(2) INFORMATION FOR SEQ ID NO: 237:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

25 (B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

30 (D) OTHER INFORMATION: Full-length reverse

complement of SEQ ID NO: 236

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 237:

TGCCCCATTT TTTTGGTTTA

20

(2) INFORMATION FOR SEQ ID NO: 238:

- 155 -

(1) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- 5 (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to
Respiratory Syncytial Virus

10 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO: 3)

(I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 238:

15 AAATGGGGCA AATAAGAATT

20

(2) INFORMATION FOR SEQ ID NO: 239:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- 20 (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
25 complement of SEQ ID NO: 238

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 239:

AATTCTTATT TGCCCCATT

20

(2) INFORMATION FOR SEQ ID NO: 240:

(i) SEQUENCE CHARACTERISTICS:

- 30 (A) LENGTH: 20 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

35 (ix) FEATURE:

- 156 -

(D) OTHER INFORMATION: Antisense to
Respiratory Syncytial Virus

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: WO 95/22553 (SEQ ID NO: 4)

5 (I) FILING DATE: 17-FEB-1995

(J) PUBLICATION DATE: 24-AUG-1995

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 240:

AAAAATGGGG CAAATAAATC

20

(2) INFORMATION FOR SEQ ID NO: 241:

10 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: Nucleic Acid

(C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

15 (iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 240

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 241:

20 GATTATTTG CCCCATTTT

20

(2) INFORMATION FOR SEQ ID NO: 242:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

(B) TYPE: Nucleic Acid

25 (C) STRANDEDNESS: Single

(D) TOPOLOGY: Linear

(iv) ANTI-SENSE: Yes

(ix) FEATURE:

(D) OTHER INFORMATION: Antisense to
cytomegalovirus

30 (x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: US 5442049 (SEQ ID NO: 22)

(I) FILING DATE: 25-JAN-1993

(J) PUBLICATION DATE: 15-AUG-1995

35 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 242:

- 157 -

GCCTTGCTC TTCTTCTTGC G

21

(2) INFORMATION FOR SEQ ID NO: 243:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 21 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(iv) ANTI-SENSE: No

(ix) FEATURE:

(D) OTHER INFORMATION: Full-length reverse
complement of SEQ ID NO: 242

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 243:

CGCAAGAAGA AGAGCAAACG C

21

- 158 -

What is claimed is:

1. A matrix comprising a support and an affinity unit, wherein said affinity unit specifically and reversibly binds a target oligonucleotide, and wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide.
2. The matrix of claim 1 further comprising a linker.
3. The matrix of claim 1 further comprising a spacer.
- 10 4. The matrix of claim 1 further comprising a spacer and a linker.
5. The matrix of claim 1 wherein said support is a solid support.
- 15 6. The matrix of claim 1 wherein said support is a soluble support.
7. The matrix of claim 1 wherein said affinity unit is synthesized *in situ*.
8. The matrix of claim 1 wherein said affinity unit is synthesized independently from said support and 20 subsequently attached to said support.
9. The matrix of claim 1 wherein said affinity unit comprises at least one modified nucleobase.
10. The matrix of claim 9, wherein said modified nucleobase is selected from the group consisting of 5-methyl 25 cytosine, uridine 5-propynyl methylthiazole, thymidine 5-propynyl methylthiazole, uridine 5-amino-ethyl-3-acrylimido, thymidine 5-amino-ethyl-3-acrylimido, 2-thio uridine, 2-thio

- 159 -

thymidine, a 7-modified-7-deaza purine, and 2-amino-adenosine.

11. A matrix comprising a support and an affinity unit, wherein said affinity unit specifically and reversibly binds a target oligonucleotide, wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide, and wherein said affinity unit comprises at least one modified sugar residue.

10 12. The matrix of claim 11, wherein said modified sugar residue is a 4'-6' methano carbocyclic derivative.

13. The matrix of claim 11, wherein said modified sugar residue is a ribose residue comprising a 2' modification.

15 14. The matrix of claim 13, wherein said 2' modification is selected from the group consisting of 2'-fluoro; 2'-O-alkyl; 2'-O-allyl; 2'-O-butyl; 2'-O-methyl; 2'-O-methoxy-ethyl; 2'-O-alkoxy-alkoxy; 2'-O-aminoalkyl; an intercalating agent linked to the 2' position; and 2'-O-CH₂-CHR-X, where X = OH, F, CF₃ or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃.

15. The matrix of claim 1 wherein said affinity unit comprises at least one modified backbone linkage.

16. The matrix of claim 15, wherein said modified backbone linkage is selected from the group consisting of a polyamide backbone linkage; a methylene(methylimino) backbone linkage; a dimethylhydrazino backbone linkage; an amide 3 backbone linkage; an amide 4 backbone linkage; a phosphoryl linked morpholino backbone linkage; a phosphonate backbone linkage; a formacetal/ketal type backbone linkage; an N3'-->P5' phosphoramidite backbone linkage; and a backbone

- 160 -

linkage comprising 1,5-anhydrohexitol.

17. A matrix comprising a support and an affinity unit, wherein said affinity unit specifically and reversibly binds a target oligonucleotide, wherein said affinity unit 5 comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide, and wherein said affinity unit is or comprises a peptide nucleic acid.
18. The matrix of claim 1 wherein said affinity unit 10 comprises at least one modified sugar residue and at least one modified nucleobase.
19. The matrix of claim 18, wherein said modified sugar residue is a 4'-6' methano carbocyclic derivative.
20. The matrix of claim 18, wherein said modified 15 sugar residue is a ribose residue comprising a 2' modification.
21. The matrix of claim 20, wherein said 2' modification is selected from the group consisting of 2'-fluoro; 2'-O-alkyl; 2'-O-allyl; 2'-O-butyl; 2'-O-methyl; 20 2'-O-methoxy-ethyl; 2'-O-alkoxy-alkoxy; 2'-O-aminoalkyl; an intercalating agent linked to the 2' position; and 2'-O-CH₂-CHR-X, where X = OH, F, CF₃, or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃.
22. The matrix of claim 18, wherein said modified 25 nucleobase is selected from the group consisting of 5-methyl cytosine, uridine 5-propynyl methylthiazole, thymidine 5-propynyl methylthiazole, uridine 5-amino-ethyl-3-acrylimido, thymidine 5-amino-ethyl-3-acrylimido, 2-thio uridine, 2-thio thymidine, a 7-modified-7-deaza purine, and 2-amino-30 adenine.

- 161 -

23. The matrix of claim 1 wherein said affinity unit comprises at least one modified sugar residue and at least one modified backbone linkage.

24. The matrix of claim 23, wherein said modified sugar residue is a 4'-6' methano carbocyclic derivative.

25. The matrix of claim 23, wherein said modified sugar residue is a ribose residue comprising a 2' modification.

26. The matrix of claim 25, wherein said 2' modification is selected from the group consisting of 2'-fluoro; 2'-O-alkyl; 2'-O-allyl; 2'-O-butyl; 2'-O-methyl; 2'-O-methoxy-ethyl; 2'-O-alkoxy-alkoxy; 2'-O-aminoalkyl; an intercalating agent linked to the 2' position; and 2'-O-CH₂-CHR-X, where X = OH, F, CF₃ or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃.

27. The matrix of claim 23, wherein said modified backbone linkage is selected from the group consisting of a polyamide backbone linkage; a methylene(methylimino) backbone linkage; a dimethylhydrazino backbone linkage; an amide 3 backbone linkage; an amide 4 backbone linkage; a phosphoryl linked morpholino backbone linkage; a phosphonate backbone linkage; a formacetal/ketal type backbone linkage; an N3'→P5' phosphoramidite backbone linkage; and a backbone linkage comprising 1,5-anhydrohexitol.

28. The matrix of claim 1 wherein said affinity unit comprises at least one modified sugar residue, at least one modified backbone linkage, and at least one modified nucleobase.

29. The matrix of claim 1 wherein said affinity unit is RNA or RNA-like.

- 162 -

30. The matrix of claim 1 wherein said affinity unit comprises at least one modification selected from the group consisting of a modified sugar residue, a modified backbone linkage, and a modified nucleobase, wherein said 5 modification enhances the affinity of the affinity unit for, and/or duplex stability of the affinity unit with, RNA and RNA-like molecules.

31. The matrix of claim 1 wherein said affinity unit comprises at least one modification selected from the group 10 consisting of a modified sugar residue, a modified backbone linkage, and a modified nucleobase, wherein said modification results in said affinity unit having a capacity to form a stable duplex with an RNA or RNA-like molecule that exceeds its ability to form a stable duplex with a DNA 15 molecule of the same sequence under comparable conditions.

32. The matrix of claim 1, wherein said affinity unit has a tendency to be organized to selectively bind complementary RNA or RNA-like molecules with high affinity and specificity.

20 33. The matrix of claim 1, wherein said affinity unit preferably forms an A-type helix with complementary RNA or RNA-like molecules.

34. The matrix of claim 1, wherein said affinity unit comprises a nucleobase sequence having from 5 to about 25 25 contiguous nucleobases derived from a sequence selected from the group consisting of SEQ ID NOS:2, 17, 19, 21, 23, 27, 29, 31, 33, 35, 37, 39, 41, 43, 25, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 30 113, 115, 117, 119, 121, 123, 125, 127, 129, 131, 133, 135, 137, 139, 141, 143, 145, 147, 149, 151, 153, 155, 157, 159, 161, 163, 165, 167, 169, 171, 173, 175, 177, 179, 182, 185, 187, 189, 191, 193, 195, 197, 199, 201, 203, 205, 207, 209,

- 163 -

211, 213, 215, 217, 219, 221, 223, 225, 227, 229, 231, 233,
235, 237, 239, 241 and 243.

35. A matrix comprising a support and an affinity unit, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity unit:

(a) specifically and reversibly binds a target oligonucleotide, wherein said target oligonucleotide has a length, n, of from 5 to 50 nucleobases; and

10 (b) has a length, p, wherein p is a positive whole number ranging from 4 to n+4; and

(c) is, over said length p, the reverse complement of the nucleobase sequence of said target oligonucleotide, provided that, in a duplex between said 15 target oligonucleotide and said nucleobase sequence of said affinity unit, neither a 5' overhang nor a 3' overhang of the duplex formed between said target oligonucleotide and said affinity unit is greater than two nucleobases.

36. A matrix comprising a support and an affinity unit, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity unit specifically and reversibly binds a target oligonucleotide, wherein said target oligonucleotide and said nucleobase sequence of said affinity unit each have a 25 length, n, of from 5 to 50 nucleobases, and said nucleobase sequence of said affinity unit is the reverse complement of the nucleobase sequence of said target oligonucleotide.

37. A matrix comprising a support and an affinity unit, wherein said affinity unit specifically and reversibly binds a target oligonucleotide, wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide, and wherein said nucleobase sequence of said affinity unit is in the form of a polymer selected from

- 164 -

the group consisting of an oligodeoxyribonucleotide, an oligoribonucleotide, a chimeric oligonucleotide and an oligonucleotide having one or more modified linkages.

38. The matrix of claim 1, wherein said affinity unit 5 selectively binds and retains said target oligonucleotide with high affinity and specificity under conditions wherein derivatives of said target oligonucleotide having one or more mismatches with the nucleobase sequence of said affinity unit are not bound and retained.

10 39. The matrix of claim 38, wherein said affinity unit has at least one modification selected from the group consisting of a modified sugar residue, a modified backbone linkage and a modified nucleobase.

15 40. The matrix of claim 38, wherein said affinity unit has two or more modification selected from the group consisting of a modified sugar residue, a modified backbone linkage and a modified nucleobase.

20 41. A method of purifying a synthetic target oligonucleotide from a mixture which comprises said target oligonucleotide and one or more undesired contaminants comprising contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker; wherein said affinity unit specifically and 25 reversibly binds a target oligonucleotide; and wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide.

30 42. The method of claim 41 further comprising the step of dissociating said target oligonucleotide from said affinity unit and recovering said target oligonucleotide.

- 165 -

43. A method of purifying a synthetic target oligonucleotide from a mixture which comprises said target oligonucleotide and one or more undesired contaminants comprising contacting said mixture with a matrix, wherein 5 said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker; wherein said affinity unit specifically and reversibly binds a target oligonucleotide; wherein said affinity unit comprises a nucleobase sequence having the 10 reverse complement of a hybridizing portion of said target oligonucleotide; and wherein said nucleobase sequence of said affinity unit is at least partially in the form of a peptide nucleic acid.

44. The method of claim 43 further comprising the step 15 of dissociating said target oligonucleotide from said affinity unit and recovering said target oligonucleotide.

45. The method of claim 44 wherein said dissociating of said target oligonucleotide from said affinity unit is achieved by the addition of ammonium hydroxide.

20 46. A method of purifying a synthetic target oligonucleotide from a mixture which comprises said target oligonucleotide and one or more undesired contaminants comprising contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and 25 optionally comprises a linker, a spacer or a spacer and a linker, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity unit:

(a) specifically and reversibly binds a target 30 oligonucleotide, wherein said target oligonucleotide has a length, n, of from 5 to 50 nucleobases; and
(b) has a length, p, wherein p is a positive whole number ranging from 4 to n+4; and
(c) is, over said length p, the reverse

- 166 -

complement of the nucleobase sequence of said target oligonucleotide, provided that, in a duplex between said target oligonucleotide and said nucleobase sequence of said affinity unit, neither a 5' overhang nor a 3' overhang of 5 the duplex formed between said target oligonucleotide and said affinity unit is greater than two nucleobases.

47. A method of purifying a synthetic target oligonucleotide from a mixture which comprises said target oligonucleotide and one or more undesired contaminants 10 comprising contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity 15 unit specifically and reversibly binds a target oligonucleotide, wherein said target oligonucleotide and said nucleobase sequence of said affinity unit each have a length, n, of from 5 to 50 nucleobases, and said nucleobase sequence of said affinity unit is the reverse complement of 20 the nucleobase sequence of said target oligonucleotide.

48. A method of purifying a synthetic target oligonucleotide from a mixture comprising said target oligonucleotide and one or more undesired contaminants, comprising the steps of:

25 (a) contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said affinity unit specifically and reversibly binds a target oligonucleotide, and wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a portion of said target oligonucleotide;

30 (b) removing said undesired contaminants of said

- 167 -

target oligonucleotide; and

(c) dissociating said target oligonucleotide from said matrix and recovering said dissociated target oligonucleotide.

5 49. A method of purifying a synthetic target oligonucleotide from a mixture comprising said target oligonucleotide and one or more undesired contaminants, comprising the steps of:

10 (a) contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity unit:

15 (1) specifically and reversibly binds a target oligonucleotide, wherein said target oligonucleotide has a length, n, of from 5 to 50 nucleobases;

20 (2) has a length, p, wherein p is a positive whole number ranging from 4 to n+4; and

25 (3) is, over said length p, the reverse complement of the nucleobase sequence of said target oligonucleotide, provided that, in a duplex between said target oligonucleotide and said nucleobase sequence of said affinity unit, neither a 5' overhang nor a 3' overhang of said target oligonucleotide is greater than two nucleobases;

30 (b) removing said undesired contaminants of said target oligonucleotide; and

35 (c) dissociating said target oligonucleotide from said matrix and recovering said dissociated target oligonucleotide.

- 168 -

50. A method of purifying a synthetic target oligonucleotide from a mixture comprising said target oligonucleotide and one or more undesired contaminants, comprising the steps of:

5 (a) contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said affinity unit comprises a nucleobase sequence, wherein said nucleobase sequence of said affinity unit specifically and reversibly binds said target oligonucleotide, wherein said target oligonucleotide and said nucleobase sequence of said affinity unit each have a length, n, of from 5 to 50 nucleobases, and wherein said nucleobase sequence of said affinity unit is the reverse complement of the nucleobase sequence of said target oligonucleotide;

10 (b) removing said undesired contaminants of said target oligonucleotide; and

15 (c) dissociating said target oligonucleotide from said matrix and recovering said dissociated target oligonucleotide.

20 51. The method of claim 50, wherein said step (b) is achieved by washing said matrix.

25 52. The method of claim 50, wherein said step (b) is achieved by isocratic, gradient or step-gradient washing using appropriate buffers such that said washing begins at a pH of about 5 and concludes at a pH of about 10.

30 53. The method of claim 50, wherein said contaminant removing step (b) is performed at a temperature which is at least 1°C below the T_m of the duplex composed of said nucleobase sequence of said affinity unit hybridized to said

target oligonucleotide.

54. The method of claim 50, wherein said dissociation and recovery step (c) is achieved by washing said matrix with distilled water and collecting the resultant eluent.

5 55. A method of purifying a synthetic target oligonucleotide from a first mixture comprising said target oligonucleotide and one or more undesired contaminants, comprising the steps of contacting said first mixture to a first matrix, wherein said first matrix comprises a support 10 and a first affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said first affinity unit comprises a nucleobase sequence having the reverse complement of a first hybridizing portion of said target oligonucleotide; dissociating and recovering 15 oligonucleotide material bound to said matrix comprising said first affinity unit, to obtain a second mixture comprising said target oligonucleotide and one or more undesired contaminants bound by said first affinity unit; contacting said second mixture to a second matrix, wherein 20 said second matrix comprises a support and a second affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker, wherein said second affinity unit comprises a nucleobase sequence having the reverse complement of a second hybridizing portion of said target 25 oligonucleotide; dissociating and recovering oligonucleotide material bound to said second matrix comprising said second affinity unit, to obtain a third mixture comprising said target oligonucleotide; wherein said first and second hybridizing portions of said target oligonucleotide are 30 distinct enough from one another such that different undesirable contaminating derivatives of the target oligonucleotide are removed by said first and said second matrix.

56. The method of claim 55, wherein said nucleobase

- 170 -

sequence of said affinity unit is in the form of a polymer selected from the group consisting of a peptide nucleic acid, an oligodeoxyribonucleotide, an oligoribonucleotide, a chimeric oligonucleotide and an oligonucleotide having one 5 or more modified linkages.

57. The method of claim 55, wherein said one or more undesired contaminants comprises one or more deletion derivatives of said target oligonucleotide.

58. The method of claim 41, wherein said target 10 oligonucleotide is a chimeric oligonucleotide.

59. The method of claim 41, wherein said target oligonucleotide is or comprises a peptide nucleic acid.

60. A method of purifying a synthetic target oligonucleotide from a mixture which comprises said target 15 oligonucleotide and one or more undesired contaminants comprising contacting said mixture with a matrix, wherein said matrix comprises a support and an affinity unit, and optionally comprises a linker, a spacer or a spacer and a linker; wherein said affinity unit specifically and 20 reversibly binds a target oligonucleotide; wherein said affinity unit comprises a nucleobase sequence having the reverse complement of a hybridizing portion of said target oligonucleotide; and wherein said target oligonucleotide is an oligonucleotide designed to have antisense activity.

25 61. The method of claim 60, wherein said target oligonucleotide has an antisense sequence to a portion of a gene selected from the group consisting of *c-myb*, *bcl-2*, *bcl-abl*, *c-raf*, a gene encoding a protein kinase C, and a gene encoding a growth factor.

30 62. The method of claim 61, wherein said target oligonucleotide is an oligonucleotide designed to have

- 171 -

therapeutic activity against disorders resulting at least in part from hyperproliferative cells.

63. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have 5 therapeutic activity against a non-pathogenic and non-hyperproliferative disorder.

64. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to modulate the expression of a protein displayed on a cell surface.

10 65. The method of claim 64, wherein said protein selected on a cell surface is selected from the group consisting of ICAM-1, ICAM-2, ICAM-3, VCAM, a B7 protein, and an MDR P-glycoprotein.

15 66. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against a eukaryotic pathogen.

67. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against a human retrovirus.

20 68. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against a human immunodeficiency virus.

69. The method of claim 68, wherein said target oligonucleotide has an antisense sequence to a retroviral 25 gene selected from the group consisting of gag, tat, vpr, rev, env, nef and pol.

70. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against a virus other than a human

- 172 -

retrovirus.

71. The method of claim 70, wherein said virus other than a human retrovirus is selected from the group consisting of influenza virus, Epstein-Barr virus, 5 Respiratory Syncytial Virus, and cytomegalovirus.

72. The method of claim 60, wherein said antisense oligonucleotide has an antisense sequence to a portion of a gene selected from the group consisting of *c-myb*, *bcl-2*, *bcl-abl*, *c-raf*, a gene encoding a protein kinase C, and a 10 gene encoding a growth factor.

73. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against disorders resulting at least in part from hyperproliferative cells.

15 74. The method of claim 60, wherein said target oligonucleotide is an oligonucleotide designed to have therapeutic activity against a non-pathogenic and non-hyperproliferative disorder.

75. The method of claim 41, wherein said hybridizing 20 portion of said target oligonucleotide is RNA or RNA-like, and said affinity unit comprises at least one of the following modifications: at least one modified sugar residue, at least one modified backbone linkage, or a modified nucleobase.

25 76. The method of claim 75, wherein said affinity unit comprises at least one ribose residue comprising a 2' modification.

77. The method of claim 76, wherein said 2' modification is selected from the group consisting of 2'-30 fluoro; 2'-O-'alkyl; 2'-O-allyl; 2'-O-butyl; 2'-O-methyl;

- 173 -

2'-O-methoxy-ethyl; 2'-O-alkoxy-alkoxy; 2'-O-aminoalkyl; an intercalating agent linked to the 2' position; and 2'-O-CH₂-CHR-X, where X = OH, F, CF₃ or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃.

5 78. The method of claim 75, wherein said affinity unit comprises at least one modified backbone linkage, wherein said modified backbone linkage is selected from the group consisting of a polyamide backbone linkage; a methylene(methylenimino) backbone linkage; a dimethylhydrazino 10 backbone linkage; an amide 3 backbone linkage; an amide 4 backbone linkage; a phosphoryl linked morpholino backbone linkage; a phosphonate backbone linkage; a formacetal/ketal type backbone linkage; an N3'->P5' phosphoramidite backbone linkage; and a backbone linkage comprising 1,5- 15 anhydrohexitol.

79. The method of claim 75, wherein said affinity unit comprises at least one modified nucleobase, wherein said modified nucleobase is selected from the group consisting of 5-methyl cytosine, uridine 5-propynyl methylthiazole, 20 thymidine 5-propynyl methylthiazole, uridine 5-amino-ethyl-3-acrylimido, thymidine 5-amino-ethyl-3-acrylimido, 2-thio uridine, 2-thio thymidine, a 7-modified-7-deaza purine, and 2-amino-adenosine.

80. The method of claim 75, wherein said affinity unit 25 comprises at least two of the following elements:
(a) at least one modified backbone linkage selected from the group consisting of a polyamide backbone linkage; a methylene(methylenimino) backbone linkage; a dimethylhydrazino backbone linkage; an amide 3 backbone linkage; an amide 4 backbone linkage; a phosphoryl linked morpholino backbone linkage; a phosphonate backbone linkage; a formacetal/ketal type backbone linkage; an N3'->P5' phosphoramidite backbone linkage; and a backbone linkage comprising 1,5-anhydrohexitol;

(b) at least one ribose residue comprising a 2' modification selected from the group consisting of 2'-fluoro, 2'-O-alkyl, 2'-O-allyl, 2'-O-butyl, 2'-O-methyl, 2'-O-methoxy-ethyl, 2'-O-alkoxy-alkoxy, 2'-O-aminoalkyl, an 5 intercalating agent linked to the 2' position, and 2'-O-CH₂-CHR-X, where X = OH, F, CF₃, or OCH₃, and R = H, CH₃, CH₂OH or CH₂OCH₃, and

(c) at least one modified nucleobase selected from the group consisting of 5-methyl cytosine, uridine 5- 10 propynyl methylthiazole, thymidine 5-propynyl methylthiazole, uridine 5-amino-ethyl-3-acrylimido, thymidine 5-amino-ethyl-3-acrylimido, 2-thio uridine, 2-thio thymidine, a 7-modified-7-deaza purine, and 2-amino-adenosine.

15 81. The method of claim 41, wherein said hybridizing portion of said target oligonucleotide is RNA or RNA-like, and said affinity unit has a tendency to be organized to selectively bind complementary RNA or RNA-like molecules with high affinity and specificity.

20 82. The method of claim 41, wherein said hybridizing portion of said target oligonucleotide is RNA or RNA-like, and said affinity unit preferably forms an A-type helix with complementary RNA or RNA-like molecules.

83. The method of claim 41, wherein said target 25 oligonucleotide comprises at least one ribose residue comprising a 2' modification, or at least one 5-methyl cytosine, in said hybridizing portion thereof, and said affinity unit comprises at least one of the following modifications: at least one modified sugar residue, at least 30 one modified backbone linkage, or at least one modified nucleobase.

84. The method of claim 41, said affinity unit having at least one ribose residue comprising a 2' modification.

- 175 -

85. The method of claim 41, wherein said hybridizing portion of said target oligonucleotide comprises at least one of the following modifications: at least one modified sugar residue, at least one modified backbone linkage, or at 5 least one modified nucleobase, and said affinity unit is RNA or RNA-like.

86. The method of claim 41 wherein said target oligonucleotide has been prepared by a blockwise synthesis procedure.

10 87. The method of claim 41 wherein said target oligonucleotide has been prepared by solution synthesis.

88. A target oligonucleotide enriched in purity by the method of claim 41.

15 89. A target oligonucleotide enriched in purity by the method of claim 43.

90. A target oligonucleotide designed to have antisense activity enriched in purity by the method of claim 60.

91. A target oligonucleotide comprising at least one 20 ribose residue comprising a 2' modification or at least one 5-methyl cytosine enriched in purity by the method of claim 79.

92. A pharmaceutical composition comprising a target oligonucleotide enriched in purity by the method of claim 25 41.

93. A pharmaceutical composition comprising a target oligonucleotide enriched in purity by the method of claim 43.

- 176 -

94. A pharmaceutical composition comprising a target oligonucleotide designed to have antisense activity and enriched in purity by the method of claim 60.

95. A pharmaceutical composition comprising a target oligonucleotide enriched in purity by the method of claim 79, wherein said target oligonucleotide comprises at least one ribose residue comprising a 2' modification or at least one 5-methyl cytosine.

1/4

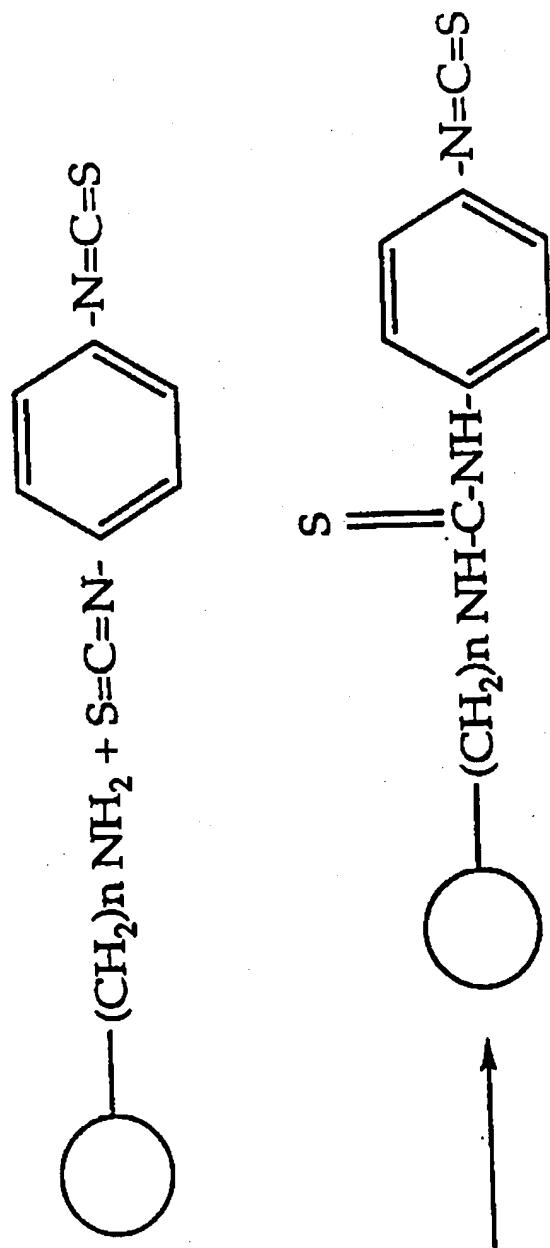


Figure 1

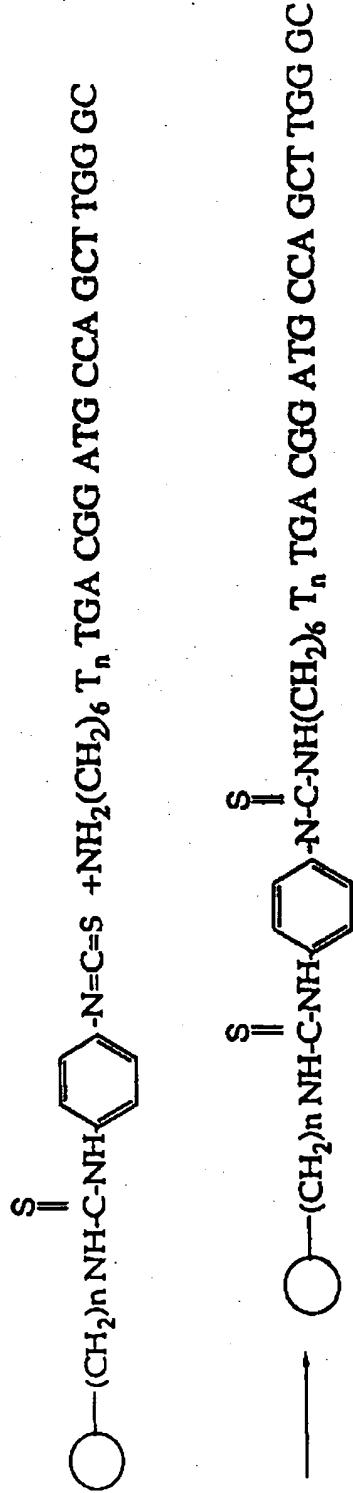
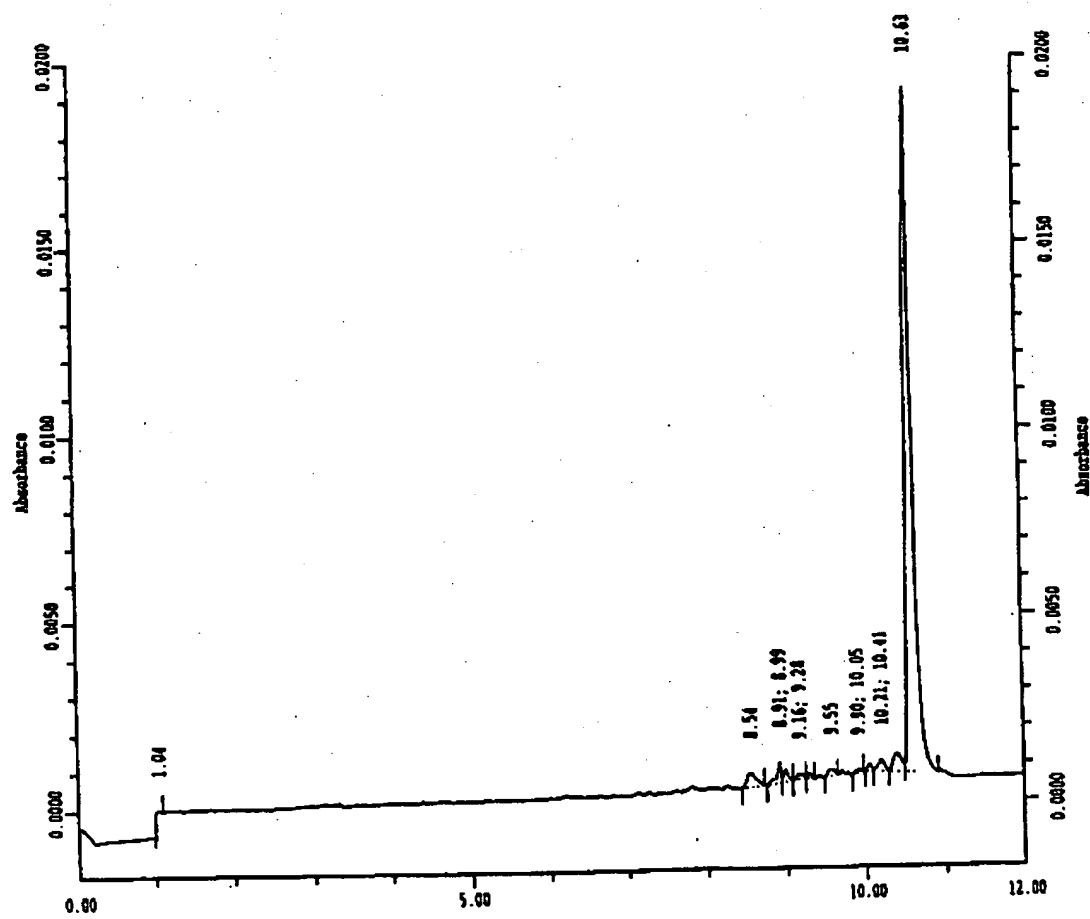
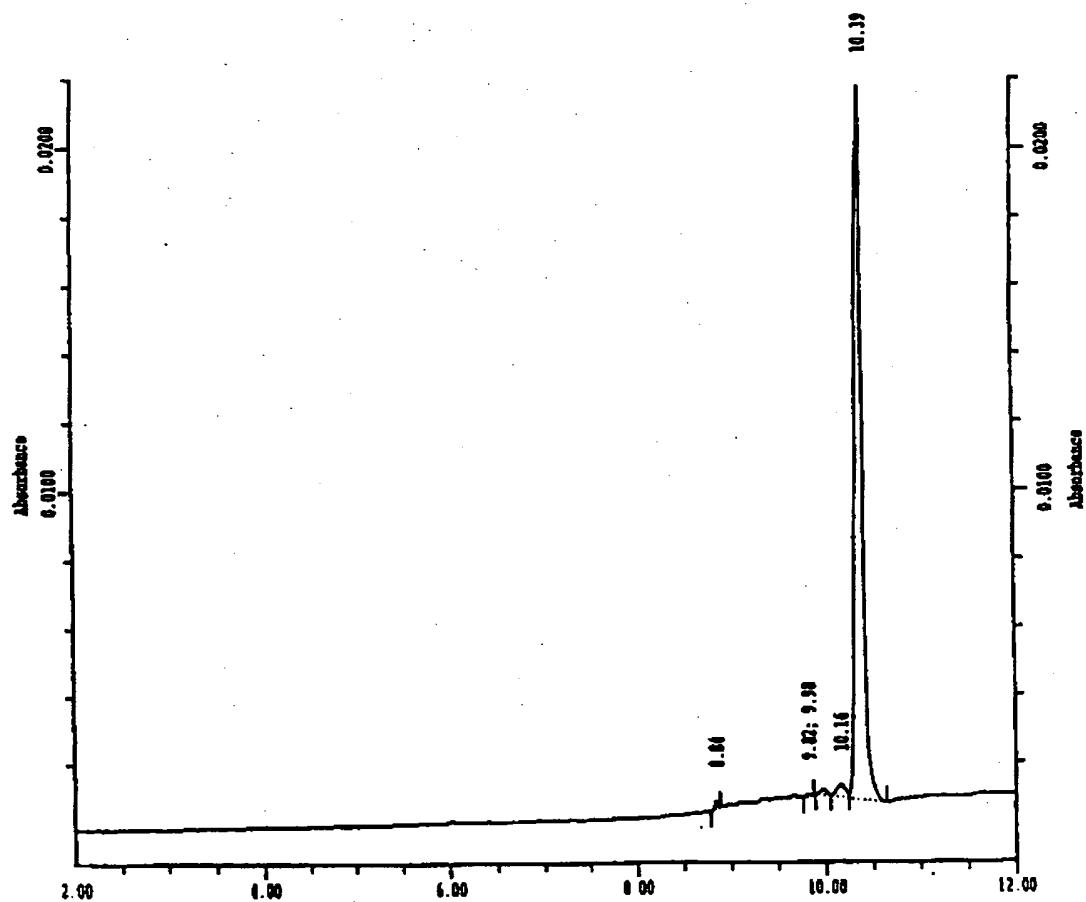


Figure 2

3/4

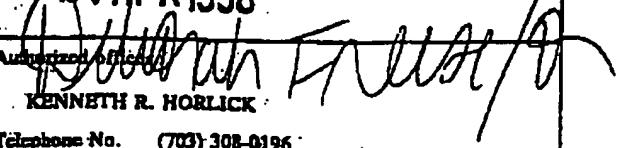
**Figure 3**

4/4

**Figure 4**

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23284

| | | |
|--|---|---|
| A. CLASSIFICATION OF SUBJECT MATTER | | |
| IPC(6) : G01N 33/48; C07H 21/04 US CL : 422/104; 436/94; 536/25.4, 24.5, 24.3 According to International Patent Classification (IPC) or to both national classification and IPC | | |
| B. FIELDS SEARCHED | | |
| Minimum documentation searched (classification system followed by classification symbols) U.S. : 422/104; 436/94; 536/25.4, 24.5, 24.3 | | |
| Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched | | |
| Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) APS, EIOSIS, MEDLINE, DERWENT, CA search terms: support, purify, oligonucleotides, separate, full length, deletion, elute | | |
| C. DOCUMENTS CONSIDERED TO BE RELEVANT | | |
| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | GOSS et al. High performance affinity chromatography of DNA. J. Chromatog. 1990, Vol. 508, pages 279-287, especially pages 279, 283, and 285. | 88-90 |
| Y | YASHIMA et al. High-performance affinity chromatography of oligonucleotides on nucleic acid analogue immobilized silica gel columns. J. Chromatog. 1992, Vol. 603, pages 111-119, especially pages 111 and 115-117. | 1-87 and 91-95 |
| Y | TEMSAMANI et al. Sequence identity of the n-1 product of a synthetic oligonucleotide. Nucleic Acids Res. 1995, Vol. 23, No. 11, pages 1841-1844, especially page 1841. | 1-95 |
| <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex. | | |
| <p><input checked="" type="checkbox"/> <i>General categories of cited documents:</i></p> <p>*A* document defining the general state of the art which is not considered to be of particular relevance</p> <p>*B* other document published on or after the international filing date</p> <p>*C* document which may throw doubt on priority claim(s) or which is used to establish the publication date of another citation or other special reasons (as specified)</p> <p>*D* document referring to an oral disclosure, use, exhibition or other events</p> <p>*E* document published prior to the international filing date but later than the priority date claimed</p> | | <p>*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>*A* document member of the same patent family</p> |
| Date of the actual completion of the international search 01 APRIL 1998 | Date of mailing of the international search report 27 APR 1998 | |
| Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230 | <p>Authorized officer KENNETH R. HORLICK</p> <p>Telephone No. (703) 308-0196</p>  | |

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/23284

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| Y | SAIKI et al. Analysis of enzymatically amplified B-globin and HLA-DQ DNA with allele-specific oligonucleotide probes. Nature. 13 November 1986, Vol. 324, pages 163-166, especially page 163. | 1-95 |